NTP REPORT

ON THE

TOXICOLOGY STUDIES OF DICHLOROACETIC ACID
(CAS NO. 79-43-6)

IN GENETICALLY MODIFIED (FVB Tg.AC HEMIZYGOUS) MICE

(DERMAL AND DRINKING WATER STUDIES)

AND CARCINOGENICITY STUDIES OF DICHLOROACETIC ACID

IN GENETICALLY MODIFIED [B6.129-*Trp53*^{tm1Brd} (N5) HAPLOINSUFFICIENT] MICE

(DRINKING WATER STUDIES)

Scheduled Peer Review Date: September 27-28, 2005

NTP GMM 11

NIH Publication No. 05-4428

National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

The studies described in this Report series were designed and conducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies, abstracts of all NTP Reports, and full versions of the the completed reports are available at the NTP's World Wide Web site: http://ntp.niehs.nih.gov. In addition, printed copies of these reports are available from the NTP as supplies last by contacting (919) 541-1371.

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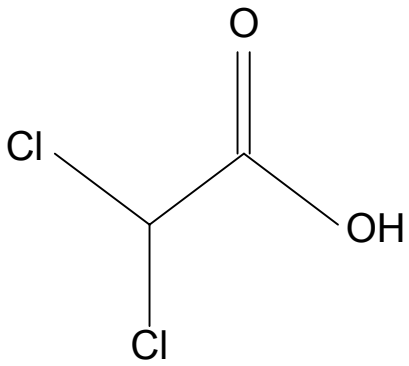
ABSTRACT



DICHLOROACETIC ACID

CAS No. 79-43-6

Chemical Formula: $C_2H_2Cl_2O_2$ Molecular Weight: 128.9426

Synonyms: Acetic acid, dichloro; bichloracetic acid; DCA; dichlorethanoic acid; 2,2-dichloroacetic acid; dichloroethanoic acid; kyselina dichloroctova; Urner's liquid

Dichloroacetic acid was nominated for study by the Environmental Protection Agency (EPA) and by the National Institute of Environmental Health Sciences (NIEHS) because of its widespread occurrence in drinking water as a by-product of water disinfection using chlorination. It was also nominated because dichloroacetic acid is the most studied representative of the class of haloacetic acids and has been shown to cause liver tumors in both rats and mice. Haloacetic acids are second only to trihalomethanes as a family of disinfection by-products found in many drinking water supplies. Dichloroacetic acid is one of several disinfection by-products being evaluated to determine whether genetically modified mouse models can serve as a more rapid and cost-effective means of evaluating and ranking potential hazards of disinfection by-products.

The NTP has explored the use of genetically altered mouse models as adjuncts to 2-year rodent cancer assays. These models may prove to be more rapid, use fewer animals, and provide some mechanistic insights into neoplastic responses. As part of the evaluation of new mouse cancer screening models, dichloroacetic acid was

tested for potential toxicity and carcinogenicity in two relatively well-studied models, the Tg.AC hemizygous strain and the p53 haploinsufficient strain. Male and female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to dichloroacetic acid in the drinking water (greater than 98% pure) for 26 or 41 weeks. In addition, male and female Tg.AC hemizygous mice were exposed by dermal application for 26 or 39 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium* strains TA98, TA100, and TA1535 and in mouse peripheral blood erythrocytes.

26- and 39-Week Dermal Studies in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 31.25, 125, or 500 mg dichloroacetic acid/kg body weight 5 days per week for 26 weeks with additional groups of 10 males and 10 females continued on treatment for 39 weeks. Survival of dosed males and females was similar to that of the vehicle control groups for both studies. Mean body weights of dosed females were generally greater than those of the vehicle controls after approximately week 20 of the 26-week study. The mean body weights of the 500 mg/kg females were greater after week 17 of the 39-week study, and those of 31.5 and 125 mg/kg females were greater at the end of the study. In the 39-week study, mean body weights of 31.25 and 500 mg/kg males were less than those of the vehicle controls after approximately 22 weeks. The absolute liver weights were increased by greater than fifty percent compared to the vehicle controls for the 500 mg/kg males and females in both studies. At the site of application, the incidences of squamous cell papilloma were significantly increased in 500 mg/kg males and females at 39 weeks, and one 125 mg/kg male and two 500 mg/kg males and females had squamous cell papillomas at 26 weeks. The incidences of epidermal hyperplasia and hyperkeratosis were significantly increased in 125 and 500 mg/kg males at 26 and 39 weeks and 125 and 500 mg/kg females at 39 weeks. There was a modest increase in pulmonary adenomas in males and females exposed to 125 and 500 mg/kg that may have been related to the dichloroacetic acid exposure. In both studies, there was a dose-related increase in the mean severity of hepatocyte cytoplasmic vacuolization in males and females, and the incidence of nephropathy was increased in 500 mg/kg males.

26- and 41-Week Drinking Water Studies in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were exposed to drinking water containing 0, 500, 1,000, or 2,000 mg/L dichloroacetic acid for 26 weeks with additional groups of 10 males and 10 females exposed for 41 weeks. The equivalent average daily doses were approximately 75, 145, and 235 mg dichloroacetic acid/kg body weight to males and approximately 100, 185, and 280 mg/kg to females. Survival of exposed males was similar in both studies. In the females, survival was decreased in the 26-week but not the 41-week study. The mean body weights of 500 and 1,000 mg/L males were greater than those of the control group and those of 1,000 and 2,000 mg/L females were less than the control group in the 26-week study. In the 41-week study, mean body weights of exposed males and females tended to be less than those of the control groups. Water consumption by males and females exposed to 1,000 and 2,000 mg/L was less than that by the controls throughout both studies. The incidence and/or severity of hepatocyte cytoplasmic vacuolization was increased in both males and females in both studies. The incidence of pulmonary adenoma was increased in the male mice exposed to 1,000 mg/L dichloroacetic acid for 41 weeks. Two pulmonary adenomas were found in the 2,000 mg/L females at 41 weeks. At 26 weeks, a pulmonary carcinoma was found in one 1,000 mg/L male, one 500 mg/L female, and one 2,000 mg/L female.

26- and 41-Week Drinking Water Studies in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were exposed to drinking water containing 0, 500, 1,000, or 2,000 mg/L dichloroacetic acid for 26 weeks with additional groups of 10 males and 10 females exposed for 41 weeks. The equivalent average daily doses were approximately 45, 85, and 145 mg/kg to males and approximately 75, 145, and 220 mg/kg to females. Survival of all exposed groups was similar to that of the control groups in both studies. Mean body weights of 1,000 and 2,000 mg/L males and females were generally less than those of the control groups throughout most of both studies; mean body weights of 500 mg/L males and females were less than those of the controls for much of the 41-week study. Water consumption by 1,000 and 2,000 mg/L males and females was less than that by the control groups throughout both studies. The incidences and/or severities of hepatocyte cytoplasmic vacuolization were increased in males in the 26-week study and females in both studies.

GENETIC TOXICOLOGY

Dichloroacetic acid was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 in tests conducted in the absence of S9 liver activation enzymes; no increase in mutations was observed in either strain in the presence of rat or hamster liver S9. Dichloroacetic acid was not mutagenic in *S. typhimurium* strain TA98 with or without S9. Dichloroacetic acid was also tested for micronucleus induction in peripheral blood erythrocytes of male and female Tg.AC hemizygous and p53 haploinsufficient mice treated by drinking water or dermal application for 26 weeks. No induction of micronuclei was seen in Tg.AC hemizygous mice treated by either route or in the p53 haploinsufficient mice, which were exposed only by the drinking water route. In another study, analysis of peripheral blood samples for frequency of micronucleated erythrocytes in male and female B6C3F_1 mice exposed to dichloroacetic acid in drinking water for 3 months revealed no alteration in micronucleus frequencies in male mice; a small increase seen in females was judged to be equivocal.

CONCLUSIONS

Under the conditions of these drinking water studies, there was *no evidence of carcinogenic activity** of dichloroacetic acid in male or female p53 haploinsufficient mice exposed to 0, 500, 1,000, or 2,000 mg/L for 26 or 41 weeks. The incidences and/or severities of cytoplasmic vacuolization of the hepatocyte were increased in males and females exposed to dichloroacetic acid for 26 or 41 weeks.

Under the conditions of these dermal studies, there were increased incidences of squamous cell papillomas at the site of application in male and female Tg.AC hemizygous mice exposed to 500 mg/kg for 39 weeks. There were dose-related increased incidences of epidermal hyperkeratosis and hyperplasia at the site of application in both male and female mice exposed to dichloroacetic acid for 26 or 39 weeks.

Under the conditions of these drinking water studies, there was an increase in the incidence of alveolar/bronchiolar adenoma in male Tg.AC hemizygous mice exposed to 1,000 mg/L for 41 weeks. There were a few bronchiolar/alveolar carcinomas in males and females exposed to dichloroacetic acid in the drinking water for 26 weeks and a few bronchiolar/alveolar adenomas in females exposed to dichloroacetic acid in the drinking water for 41 weeks.

There were increased incidences and/or severities of cytoplasmic vacuolization of the hepatocyte in male and female Tg.AC hemizygous mice exposed to dichloroacetic acid in the drinking water study for 26 or 41 weeks.

The marginally increased incidences of pulmonary adenomas and/or carcinomas compared to the unexposed groups found in both the dermal and drinking water studies at 26, 39, or 41 weeks were considered to be related to dichloroacetic acid exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13.

Summary of the 26- and 39-Week Carcinogenesis and Genetic Toxicology Studies in Tg.AC Hemizygous Mice in the Dermal Studies of Dichloroacetic Acid

	Male		Female	
	26-Week	39-Week	26-Week	39-Week
Concentrations in acetone	0, 31.25, 125, or 500 mg/kg	0, 31.25, 125, or 500 mg/kg	0, 31.25, 125, or 500 mg/kg	0, 31.25, 125, or 500 mg/kg
Body weights	Dosed groups similar to the vehicle control group	31.25 and 500 mg/kg groups less than that of the vehicle control group	Dosed groups greater than the vehicle control group	Dosed groups greater than the vehicle control group
Survival rates	13/15, 14/15, 14/15, 12/15	9/10, 6/10, 8/10, 7/10	11/15, 12/15, 14/15, 15/15	8/10, 5/10, 6/10, 8/10
Nonneoplastic effects	Kidney: nephropathy (7/15, 7/15, 11/15, 13/15) Liver: hepatocyte vacuolization cytoplasmic (3/15, 4/15, 14/15, 15/15); severity of hepatocyte vacuolization cytoplasmic (1.0, 1.0, 1.8, 2.8) Skin (site of application): epidermal hyperkeratosis (2/15, 7/15, 15/15, 14/15) epidermal hyperplasia (0/15, 2/15, 11/15, 13/15)	Kidney: nephropathy (2/10, 7/10, 7/10, 8/10) Liver: hepatocyte vacuolization cytoplasmic (9/10, 7/10, 8/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (1.1, 1.3, 2.3, 3.0) Skin (site of application): epidermal hyperkeratosis (2/10, 8/10, 9/10, 10/10) epidermal hyperplasia (0/10, 0/10, 8/10, 9/10)	Liver: hepatocyte vacuolization cytoplasmic (6/15, 14/15, 14/15, 15/15); severity of hepatocyte vacuolization cytoplasmic (1.2, 1.0, 2.1, 3.3) Skin (site of application): epidermal hyperkeratosis (8/15, 9/15, 14/15, 14/15) epidermal hyperplasia (0/15, 1/15, 10/15, 13/15)	Liver: hepatocyte vacuolization cytoplasmic (7/10, 6/10, 8/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (1.0, 1.2, 2.0, 2.7) Skin (site of application): epidermal hyperkeratosis (5/10, 8/10, 9/10, 10/10) epidermal hyperplasia (0/10, 0/10, 3/10, 6/10)
Neoplastic effects	None	Skin (site of application): squamous cell papilloma (0/10, 0/10, 2/10, 8/10)	None	Skin (site of application): squamous cell papilloma (0/10, 0/10, 0/10, 6/10)
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive without S9; negative with S9 (TA100 and TA1535); negative with and without S9 (TA98)			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*				
Tg.AC 26-week dermal study	Negative in males and females			

Summary of the 26- and 41-Week Carcinogenesis and Genetic Toxicology Studies in Tg.AC Hemizygous Mice in the Drinking Water Studies of Dichloroacetic Acid

	Male		Female	
	26-Week	41-Week	26-Week	41-Week
Concentrations in water	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L
Body weights	500 and 1,000 mg/L groups greater than the control group	Exposed groups less than the control group	1,000 and 2,000 mg/L groups less than the control group	Exposed groups less than the control group
Survival rates	14/15, 13/15, 11/15, 14/15	9/10, 9/10, 10/10, 10/10	15/15, 8/15, 13/15, 10/15	7/10, 9/10, 7/10, 8/10
Nonneoplastic effects	Liver: hepatocyte vacuolization cytoplasmic (7/15, 13/15, 15/15, 15/15); severity of hepatocyte vacuolization (1.0, 1.8, 2.7, 3.7)	Liver: hepatocyte vacuolization cytoplasmic (9/10, 10/10, 9/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (2.0, 2.3, 3.2, 3.8)	Liver: hepatocyte vacuolization cytoplasmic (6/15, 10/15, 14/15, 14/15); severity of hepatocyte vacuolization (1.3, 2.7, 3.1, 3.7)	Liver: hepatocyte vacuolization cytoplasmic (7/10, 9/10, 9/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (2.0, 2.6, 2.9, 3.0)
Neoplastic effects	Lung: alveolar/bronchiolar carcinoma (0/15, 0/15, 1/15, 0/15)	Lung: alveolar/bronchiolar adenoma (1/10, 2/10, 7/10, 3/10)	Lung: alveolar/bronchiolar carcinoma (0/15, 1/15, 0/15, 1/15)	Lung: alveolar/bronchiolar adenoma (0/10, 0/10, 0/10, 2/10)
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive without S9; negative with S9 (TA100 and TA1535); negative with and without S9 (TA98)			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*				
Tg.AC 26-week drinking water study	Negative in males and females			

Summary of the 26- and 41-Week Carcinogenesis and Genetic Toxicology Studies in p53 Haploinsufficient Mice in the Drinking Water Studies of Dichloroacetic Acid

	Male		Female	
	26-Week	41-Week	26-Week	41-Week
Concentrations in water	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L	0, 500, 1,000, or 2,000 mg/L
Body weights	1,000 and 2,000 mg/L groups less than the control group	Exposed groups less than the control group	1,000 and 2,000 mg/L groups less than the control group	Exposed groups less than the control group
Survival rates	15/15/, 15/15, 15/15, 15/15	9/10, 10/10, 9/10, 10/10	15/15/, 15/15, 14/15, 14/15	10/10, 9/10, 10/10, 9/10
Nonneoplastic effects	Liver: hepatocyte vacuolization cytoplasmic (15/15, 15/15, 15/15, 15/15); severity of hepatocyte vacuolization cytoplasmic (2.7, 3.4, 3.4, 4.0)	Liver: hepatocyte vacuolization cytoplasmic (9/10, 10/10, 10/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (3.6, 3.0, 3.7, 3.8)	Liver: hepatocyte vacuolization cytoplasmic (3/15, 15/15, 15/15, 15/15); severity of hepatocyte vacuolization cytoplasmic (1.0, 2.2, 3.1, 3.5)	Liver: hepatocyte vacuolization cytoplasmic (10/10, 10/10, 10/10, 10/10); severity of hepatocyte vacuolization cytoplasmic (1.9, 2.7, 3.7, 3.6)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive without S9, negative with S9 (TA100 and TA1535); negative with and without S9 (TA98)			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*				
p53 26-week drinking water study	Negative in males and females			
B6C3F$_1$ 3-month drinking water study	Negative in males; equivocal in females			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Report on dichloroacetic acid September 27-28, 2005, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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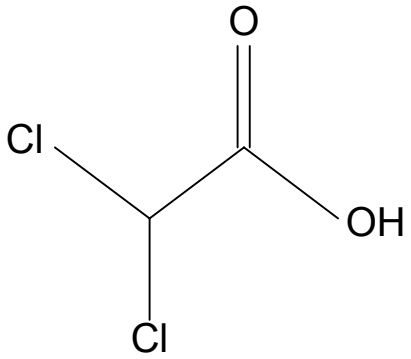
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

INTRODUCTION



DICHLOROACETIC ACID

CAS No. 79-43-6

Chemical Formula: $C_2H_2Cl_2O_2$ Molecular Weight: 128.9426

Synonyms: Acetic acid, dichloro; bichloracetic acid; DCA; dichlorethanoic acid; 2,2-dichloroacetic acid; dichloroethanoic acid; kyselina dichloroctova; Urner's liquid

Chemical and Physical Properties

Dichloroacetic acid, a clear, colorless liquid with a pungent odor and density of 1.5724 g/mL at 20° C, is formed when organic substances in water react with chlorine (Stevens *et al*., 1976; Hoehn *et al.*, 1978; Rook, 1980). Dichloroacetic acid as a chlorinated organic molecule is included in the haloacetic acids class of chemicals formed as a by-product when drinking water supplies are disinfected by chlorination (Rook, 1974). Dichloroacetic acid and trichloroacetic acid are the two most abundant haloacetic acids in most water supplies (Krasner *et al*., 1989).

Production, Use, and Human Exposure

The U.S. Environmental Protection Agency has established a maximum contaminant level of 0.060 mg/L for the five most common regulated haloacetic acids (HAA5) in community water systems serving more than 10,000 persons (40 CFR §141.64). The presence of haloacetic acids in the drinking water is believed to pose a risk

to humans because haloacetic acids have been shown to cause tumors in rats and mice following long-term exposure (Bull *et al.,* 1990; Daniel *et al.*, 1992; DeAngelo *et al.*, 1996; Komulainen, 2004). Haloacetic acids are widespread in the environment, not only in water supplies but also in swimming pools, soft drinks, and dump sites (NTP, 1987). The concentration of total haloacetic acids in chlorinated water supplies in the United States occasionally exceeds 0.1 mg/L (Weisel *et al.,* 1999). Assuming that the average daily water consumption for an adult male human weighing 70 kg is 2 liters per day, intake of dichloroacetic acid could approach a maximum daily consumption of 3.0 µg/kg per day.

Absorption, Distribution, and Excretion

Dichloroacetic acid is rapidly and completely absorbed from the stomach and intestinal tract in rodents and humans with glyoxylate, glycolate, and carbon dioxide being the major metabolites (Lin *et al.*, 1993; Gonzales-Leon *et al.*, 1997). In rats given 0.05 to 20 mg/kg either intravenously or by oral gavage, the elimination is so rapid that only doses above 1 mg/kg intravenously and 5 mg/kg orally provide plasma levels above detection limits of 6 ng/mL (Saghir and Schultz, 2002). Dichloroacetic acid exposure appears to induce CYP2E1 activity in both male and female rats (Yang *et al.*, 1996).

Toxicity

Experimental Animals

Dichloroacetic acid is relatively nontoxic; doses of greater than 4,000 mg/kg are required for an LD_{50} in fasted mice (Yount *et al.*, 1982).

Humans

In a few cases, humans have been treated with dichloroacetic acid at 25 mg/kg for as long as 5 years (Stacpoole *et al.*, 1998). Approximately 50% of the patients receiving 25 to 50 mg/kg experienced sedative effects. There have been three reported cases of peripheral neuropathy following dichloroacetic acid treatment but all were completely reversible within 6 months following cessation of treatment (Stacpoole *et al.*, 1998).

Reproductive and Developmental Toxicity

Experimental Animals

Decreased testes weights, testicular atrophy with no mature spermatozoa, and decreased spermatocytes in the seminiferous tubules were found in male Sprague-Dawley rats exposed to 1,100 mg/kg per day dichloroacetic acid in the drinking water for 90 days (Bhat *et al.*, 1991). Doses of 0, 125, 500, or 2,000 mg/kg per day of dichloroacetic acid were administered by gavage to adult rats for 3 months (Katz *et al.*, 1981). All males given 2,000 mg/kg and 40% of the males given 500 mg/kg had testicular germinal epithelial degeneration. In addition, all males given 2,000 mg/kg and 20% given 500 mg/kg had syncytial giant cells in the germinal epithelium. Morphologic changes were not found in reproductive tissues in females at any dose.

Male Long-Evans rats dosed by oral gavage with 0, 31.25, 62.5, or 125 mg/kg per day dichloroacetic acid for 10 weeks had increased relative liver weights at all doses (Toth *et al.*, 1992). The absolute preputial gland and epididymal weights, but not testes weights, were decreased at all doses. The number and motility of the sperm was affected in four of ten rats given 62 mg/kg and in nine of ten rats given 125 mg/kg.

In beagle dogs administered 12.5 to 72 mg/kg of dichloroacetic acid in capsules for 90 days, testicular lesions were observed at 12.5 mg/kg (Cicmanec *et al.*, 1991).

Humans

Because dichloroacetic acid has been used therapeutically in humans, a number of individuals have been exposed to 25 mg/kg per day or greater for years. No studies reporting reproductive effects in humans exposed to dichloroacetic acid were found in the literature. While adverse birth outcomes related to chlorinated drinking water have been suggested, emphasis has been on potential trihalomethane exposures and not exposure to haloacetic acids (Nieuwenhuijsen *et al.*, 2000).

NEUROTOXICITY

Experimental Animals

Central and peripheral neuropathy have been reported in male and female rats treated with doses as low as 125 mg/kg day of dichloroacetic acid for 10 weeks (Katz *et al.*, 1981). Severe neuropathy has been noted in male F344 rats exposed to 2.5 to 5 g/L dichloroacetic acid in the drinking water (DeAngelo *et al.*, 1996). In a series of studies involving adult and weanling Long-Evans and F344 rats of both sexes, doses as low as 16 mg/kg per day produced gait and grip strength changes predominantly in the hind limbs (Moser *et al.*, 1999). In general, dichloroacetic acid was more potent when given in the drinking water than when given by oral gavage. Severe neuromuscular toxicity induced by dichloroacetic acid exposure for 6 months was not reversible, while milder neurotoxicity induced by lower concentrations and for shorter durations of exposure was reversible. The F344 rats appeared more sensitive to neurotoxicity than did the Long-Evans hooded rats. Neurotoxicity also has been induced in Wistar rats (Yount *et al.*, 1982) and Sprague-Dawley rats (Stacpoole *et al.*, 1990). Dogs given 50 to 100 mg/kg dichloroacetic acid for 13 weeks developed hind limb weakness and vacuolization of the myelinated tracts in both the cerebellum and cerebrum (Stacpoole *et al.*, 1979).

Humans

There have been three reported cases of peripheral neuropathy following dichloroacetic acid treatment but all were completely reversible within 6 months following cessation of treatment (Stacpoole *et al.*, 1998). In one of these cases, after reversal of symptoms with cessation, dichloroacetic acid treatment was resumed at 10 to 25 mg/kg for 2 years without further evidence of neuropathy. Sixteen weeks of approximately 50 mg/kg of dichloroacetate resulted in polyneuropathy in a young male patient; the symptoms regressed with cessation of treatment. A 13-year-old female experienced peripheral neuropathy with dichloroacetic acid treatment despite concomitant thiamine medication (Kurlemann *et al.*, 1995). Because of these complications, dichloroacetic acid therapy for metabolic disorders in humans is not recommended (Stacpoole *et al.*, 1979).

CARCINOGENICITY

Experimental Animals

Dichloroacetic acid appears to cause liver tumors in mice and male rats (Komulainen, 2004). Dichloroacetic acid was administered at 2.5 g/L to male F344 rats, but due to neurotoxic effects the dose was lowered to 1 g/L after 18 weeks. Between 26 and 33 rats survived to 79 weeks and there was a marginal increase in hepatocellular carcinomas in the highest dose group (DeAngelo *et al.*, 1996). Male $B6C3F_1$ mice exposed to 1 g/L of dichloroacetic acid in the drinking water developed increased incidences of hepatocellular carcinomas and hepatocellular adenomas (Daniel *et al.*, 1992). Male and female mice given 1 or 2 g/L of dichloroacetate in the drinking water for 52 weeks developed hepatocellular adenomas and carcinomas (Bull *et al.*, 1990). An examination of the *ras* proto-oncogene activation in dichloroacetic acid-induced liver tumors in mice suggested that dichloroacetic acid provided a selective growth advantage to spontaneously occurring mutations in the *ras* oncogene (Anna *et al.*, 1994).

Humans

Exposure to chlorinated drinking water and trihalomethanes have been associated with increased rates of bladder, colon, or rectal cancer in humans (Cantor *et al.*, 1998; Hildesheim, *et al.*, 1998) Reconstructing accurate and specific disinfection by-product protracted exposure histories and modeling human exposure to drinking water disinfection by-products is difficult at best. In general, epidemiology studies tend to relate cancers to trihalomethane exposures (McGeehin *et al.*, 1993; Villanueva *et al.*, 2003; Chevrier *et al.*, 2004). No studies reporting associations between dichloroacetic acid exposure in the drinking water and increased human cancer risk were found in the literature.

GENETIC TOXICITY

The majority of published genetic toxicity studies with dichloroacetic acid indicate that the compound is a weak mutagen in bacterial and mammalian cells *in vitro*. DeMarini *et al.* (1994) reported that dichloroacetic acid induced a weak but significant increase in plaque-forming units in the Microscreen® prophage induction assay

using *Escherichia coli* prophage lambda. In addition, in *Salmonella typhimurium* strain TA100, dichloroacetic acid induced a significant increase (three to fives times) in mutant colonies with and without S9 activation, using a protocol that controlled for volatility; mutational spectra analysis showed that the alterations in the *his*G46 allele of the TA100 strain were primarily GC to AT transitions (DeMarini *et al.,* 1994). Both dichloroacetic acid and its brominated analog induced DNA damage in the *E. coli* SOS repair assay, and both compounds were reported to be mutagenic in *S. typhimurium* TA100 (Giller *et al.*, 1997); in both assays, the brominated compound was more potent than the chlorinated analog. Recently, mutation induction in *S. typhimurium* strains TA98 and TA100 exposed to dichloroacetic acid in the presence of S9 activation was reported in a study comparing the relative mutagenic and cytotoxic potencies of a series of haloacetic acids (Kargalioglu *et al.*, 2002); as in other studies, these authors reported that the brominated acetic acids investigated in their study were stronger mutagens than their chlorinated analogs. Leavitt *et al.* (1997) showed that administration of dichloroacetic acid in drinking water (1.0 or 3.5 g/L) for 60 weeks produced increases in mutant frequencies measured in the bacterial *lacI* gene in hepatocytes of transgenic male B6C3F_1 mice; these *lacI* mutations were also the result of GC to AT transitions, although some transitions and transversions at TA sites within the *lacI* gene were also reported in this study.

Results of tests for dichloroacetic acid-induced genetic damage in mammalian cells are varied, with negative, weak positive, and positive results being reported, depending upon cell type and endpoint measured. Dichloroacetic acid, over a concentration range of 100 µg/mL to 800 µg/mL, was shown to be weakly mutagenic in L5178Y/$TK^{+/-}$ mouse lymphoma cells in the absence of S9 activation and in the absence of any potentially confounding fluctuations in pH caused by the addition of acetic acid to the cell cultures (Harrington-Brock *et al.*, 1998). Dichloroacetic acid was also shown to induce chromosomal aberrations in L5178Y/$TK^{+/-}$ cells; however, no significant increases in micronuclei (surrogate indicators of numerical or structural aberrations) or aneuploidy were observed in these cells (Harrington-Brock *et al.*, 1998).

Dichloroacetic acid did not induce DNA strand breaks in Chinese hamster ovary AS52 cells, when measured by alkaline single-cell gel electrophoresis (Comet assay) (Plewa *et al.*, 2002), and administration of dichloroacetic

acid to male B6C3F$_1$ mice for 3 to 10 weeks in drinking water did not result in measurable increases in oxidative damage in hepatocytes (Parrish *et al.*, 1996). However, in this same study, dose-related increases in 8-OH-dG in nuclear DNA of hepatocytes was observed after similar treatment of male B6C3F$_1$ mice with dibromoacetic acid, confirming a stronger response in genotoxicity assays for brominated analogs compared with chlorinated ones.

Dichloroacetic acid did not induce DNA strand breaks, measured by an alkaline unwinding assay, in hepatocytes, splenocytes, or gastric epithelial cells of rats and mice treated *in vivo* or in primary cultures of rat and mouse hepatocytes; in addition, no DNA strand breaks were induced in human lymphoblastic leukemia cells treated *in vitro* with dichloroacetic acid (Chang *et al.*, 1992).

Experiments in which dichloroacetic acid (0.5, 1.0, 2.0, or 3.5 g/L) was administered in drinking water to groups of male B6C3F$_1$ mice for varying periods of time from 9 days to 31 weeks resulted in a small but statistically significant ($P<0.05$) dose-related increase in micronucleated polychromatic erythrocytes at the 9-day time point, but not after 28 days of exposure (Fuscoe *et al.*, 1996). Additionally, treatment with 3.5 g/L of dichloroacetic acid for 10, 26, or 31 weeks induced a small but significant ($P\leq0.02$) increase in micronucleated normochromatic erythrocytes (mature erythrocytes, in steady state in peripheral blood by about 4 weeks of continuous treatment) measured 31 weeks after the initiation of exposure (the 10- and 26-week treatments were stop-exposure studies). Furthermore, in these same studies, it was shown that concurrent administration of vitamin E with dichloroacetic acid did not alter induction of micronuclei in erythrocytes of exposed mice and thus, the authors concluded that the cytogenetic effects of dichloroacetic acid were probably not the result of oxidative damage. Additionally, DNA migration, measured by the Comet assay, was reduced ($P=0.023$) in blood leukocytes of male B6C3F$_1$ mice treated with 3.5 g/L dichloroacetic acid in drinking water for 28 days, indicating the possibility of DNA crosslinking (Fuscoe *et al.*, 1996).

One of the rodent metabolites of dichloroacetic acid, glyoxylic acid, was reported to be mutagenic in *S. typhimurium* strains TA97, TA100, and TA104 without S9 activation, and mutagenic in TA102 with S9 (Sayato *et al.*, 1987).

Background on Genetically Altered Mice

Mutation and/or deletions of tumor suppressor genes or activation of proto-oncogenes can disrupt cell function and predispose an animal to cancer. In the current studies, two genetically altered mouse models with either a loss of heterozygosity in a critical cancer gene (*Trp53*) or a gain of oncogene function (Ha-*ras*) were used to determine how these animals would respond to dichloroacetic acid exposure. The Tg.AC hemizygous and p53 haploinsufficient mice have been shown to be susceptible to the rapid development of cancer and are being evaluated by the National Institute of Environmental Health Sciences (NIEHS) and the NTP as models for identifying chemical toxicity and/or chemical carcinogenic processes (Tennant *et al.,* 1996; Pritchard *et al.*, 2003).

FVB/N-TgN(v-Ha-ras)Led (Tg.AC) Hemizygous Mouse Model

The Tg.AC hemizygous mouse (on an FVB/N background) was developed by Leder *et al.* (1990) by introduction via pronuclear injection of a tripartite transgene composed of the promotor of the mouse embryonic zeta-globin gene, through the v-Ha-*ras* coding sequence, with point mutation in codons 12 and 59, and an SV40 polyadenylation sequence.

The Tg.AC hemizygous transgenic mouse model has been evaluated as a reporter phenotype (skin papillomas) in response to either genotoxic or nongenotoxic carcinogens, including tumor promoters (Spalding *et al.,* 1993, 1999; Tennant *et al.,* 1999). The Tg.AC strain of mice is hemizygous for a mutant v-Ha-*ras* transgene. The model was developed by Leder *et al.* (1990) with an inducible zeta-globin promoter driving the expression of a mutated v-Ha-*ras* oncogene and is regarded as a genetically initiated model. With the exception of bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is usually transcriptionally silent until activated by certain treatments including full-thickness wounding, ultraviolet

irradiation, or exposure to some chemicals (Cannon *et al.,* 1997; Trempus *et al.,* 1998). Point mutations in the Ha-*ras* gene are believed to be early events in the induction of skin papillomas and malignancies. Topical application of carcinogens to the shaved dorsal surface of Tg.AC hemizygous mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. The oral route of administration can also generate tumor responses in the skin of Tg.AC hemizygous mice and lead to squamous cell papillomas and/or carcinomas of the forestomach. To date, the appearance of either spontaneous or induced tumors has been shown to involve transgene expression. However, the mechanism of response by the Tg.AC hemizygous mouse model to chemical carcinogens is not yet understood.

In NIEHS studies, mice are exposed beginning at 2 months of age for a total of 6 to 9 months. Cutaneous papillomas at various sites have been reported at 3.7% and 3.8% incidence in 33-week-old control male and female Tg.AC hemizygous mice, respectively (Mahler *et al.,* 1998). Cutaneous papillomas occurring at sites such as the lip, pinnae, prepuce, and vulva suggest a possible relationship to grooming and chronic irritation. Up to 32% of Tg.AC homozygous and heterozygous male or female mice can develop odontogenic tumors as early as 33 weeks (Wright *et al.,* 1995; Mahler *et al.,* 1998). A number of different tumor types occur in untreated Tg.AC hemizygous mice at an incidence of greater than 3% including odontogenic tumors, forestomach papillomas, cutaneous papillomas, alveolar-bronchiolar adenomas, salivary gland duct carcinomas, and erythroleukemia (Mahler *et al.,*1998). In the FVB mouse (the background strain for the Tg.AC hemizygous mouse), alveolar/bronchiolar neoplasms occur at 14 months of age (Mahler *et al.,* 1996).

The Tg.AC hemizygous mouse model was used in the current Report for the studies of dichloroacetic acid because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al.,* 1993; Tennant *et al.,* 1995*,* 1996; Pritchard *et al.,* 2003).

B6.129-Trp53^{tm1Brd} (N5) Mouse Model

The heterozygous B6.129-*Trp53* (N12)$^{tmlBrd(+/–)}$ mouse (on a B6.129S7 background) was developed by Donehower *et al.* (1992). A null mutation was introduced into one p53 allele by homologous recombination in murine embryonic stem cells. Insertion of a neo cassette resulted in deletion of a 450-base pair gene fragment containing 106 nucleotides of exon 5 and approximately 350 nucleotides of intron 4.

Trp53, a nuclear protein, plays an essential role in the regulation of the cell cycle, specifically in the transition from G_0 to G_1, as well as G_2 to M, and the spindle apparatus. The p53 protein is labile and exists at very low concentrations in normal cells; in DNA damaged cells or a variety of transformed cell lines, however, it is expressed in high amounts, and is believed to contribute to transformation and malignancy. The p53 protein is a DNA-binding protein containing DNA-binding, oligomerization, and transcription activation domains. Many amino acid residues may be phosphorylated or acetylated, which may determine p53 function. It is postulated to bind as a tetramer to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion or promote apoptosis, functioning as a tumor suppressor. This protein is critical to tumor suppression in humans and rodents. Mutants of p53 that fail to bind the consensus DNA binding site frequently occur in human cancers, and are unable to function as tumor suppressors. Alterations of the *Trp53* gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome.

The mouse heterozygous for a p53 null allele (+/–) has only a single functional wild-type p53 allele which provides a target for mutagens. The p53 tumor suppressor gene is one of the most common sites for mutations and gene alterations in human cancer (Harris, 1996a,b,c).

Heterozygous p53$^{(+/–)}$ mice develop normally, and like humans and other mammals, develop cancer (primarily lymphomas or sarcomas) with age, but often with decreased latency.

STUDY RATIONALE

The purpose of this study was to evaluate whether a genetically modified mouse model might be more effective or more rapid than conventional rodent bioassays for evaluating potential hazards of drinking water disinfection by-products. Hundreds of potentially hazardous chemical mixtures occur, usually at very low concentrations, in drinking water as disinfection by-products (Bull *et al.*, 1995). Since genetically modified mice may respond more rapidly or to lower exposure levels of toxicants and carcinogens, studies using these mice may prove more efficient in evaluating and ranking potential hazards of disinfection by-products.

For the past few years, the NIEHS and the NTP have been actively evaluating genetically altered strains in toxicologic testing strategies. Based on completed evaluations, three models, the Tg.AC hemizygous (v-Ha-*ras*), p53-deficient (p53 haploinsufficient), and the *ras* H2 (cHa-*ras*-transgene) mice have shown potential usefulness in identifying carcinogens (Pritchard *et al.,* 2003). This Report focuses on the Tg.AC hemizygous and p53 haploinsufficient mouse models.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Dichloroacetic Acid

Dichloroacetic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI) in two lots (05316AR and 11905BU) that were used in the 26- and 39-week dermal studies and the 26- and 41-week drinking water studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Memorial Institute (Columbus, OH) and by the study laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix G). Reports on analyses performed in support of the dichloroacetic acid studies are on file at the National Institute of Environmental Health Sciences.

Lots 05316AR (a yellow liquid) and 11905BU (a colorless liquid) were identified as dichloroacetic acid by infrared (IR) and nuclear magnetic resonance (NMR) (proton and carbon-13) spectroscopy. Lots 05316AR and 11905BU were identified as dichloroacetic acid by the study laboratory using IR. The purity of lot 05316AR was determined by high-performance liquid chromatography (HPLC) and acid functional group titration. Moisture content of lot 11905BU was determined by Karl Fischer titration. The purity of lot 11905BU was determined using HPLC and acid functional group titration and ion chromatography (IC). Purity of the bulk chemical was monitored over the course of the study; no degradation was observed.

For lot 05316AR, HPLC indicated one major peak and three impurity peaks with areas less than 1.0% of the major peak area, a combined impurity peak area of 1.20% of the major peak area, and a purity of approximately 98.8%. HPLC indicated a purity of 99.9% relative to a frozen reference standard of the same lot. Acid functional group titration indicated a purity of approximately 99.4%. The overall purity of lot 05316AR was determined to be greater than 98.8%.

For lot 11905BU, Karl Fischer titration indicated 0.06% water. HPLC indicated one major peak and no impurity peaks greater than or equal to 0.1% of the area of the major peak and a purity of 100%. Acid functional group titration indicated a purity of approximately 100.6%. IC indicated one major peak and four impurity peaks with combined peak areas of 0.77% of the major peak area. The overall purity of lot 11905BU was determined to be greater than 99%. To ensure stability the bulk chemical was stored at room temperature, protected from light in amber glass containers. Stability was monitored by the study laboratory during the 26-, 39-, and 41-week studies. No degradation of the bulk chemical was detected.

12-*O*-tetradecanoylphorbol-13-acetate

12-*O*-tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO) in one lot (48H1178) that was used in the 26-week studies in Tg.AC hemizygous mice. Lot 48H1178, a white crystalline powder, was identified as TPA by Research Triangle Institute (RTI; Research Triangle Park, NC) using IR and proton nuclear magnetic resonance (NMR) spectrometry. All spectra were consistent with the structure of TPA.

The purity of lot 48H1178 was determined by RTI using high performance liquid chromatography. This analysis indicated one major peak and one impurity peak with an area equal to approximately 0.11% of the total integrated peak area. The overall purity of lot 48H1178 was determined to be greater than 99%. The TPA formulations were shown to be stable for at least 6 months.

Acetone

USP-grade acetone was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in two lots (OG0513 and OX0312) that were used in the 26-week and 39-week dermal studies. Lots OG0513 and OX0312, clear liquids, were identified as acetone using IR spectroscopy.

The purity of lots OG0513 and OX0312 was determined using gas chromatography (GC). Analysis indicated one major peak and no impurities with areas greater than or equal to 0.1% of the major peak. The overall purity of both lots was determined to be greater than 99.9%.

Preparation and Analysis of Dose Formulations

Dermal Studies

The dose formulations were prepared every 1 to 5 weeks by mixing dichloroacetic acid with deionized water to obtain the required final concentration of dichloroacetic acid (Table G1). The dose formulations were stored at room temperature in amber glass bottles with Teflon®-lined lids and used within 35 days after formulation. TPA formulations in acetone were prepared by RTI and administered by the study laboratory.

Periodic analyses of the dose formulations of dichloroacetic acid were conducted by the study laboratory using GC. During the 26- and 39-week studies, dose formulations were analyzed five times. All 12 of the dose formulations were within 10% of the target concentrations (Table G2).

Drinking Water Studies

Dose formulations were prepared every 1 to 5 weeks by adding a specified amount of dichloroacetic acid to tap water (Table G1). TPA formulations in acetone were prepared and stored as described for the dermal studies.

Periodic analyses of the dose formulations of dichloroacetic acid were conducted by the study laboratory using HPLC. During the 26- and 41-week studies, dose formulations were analyzed four times; all 12 of the dose formulations for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target concentrations (Table G3).

STUDY DESIGNS

Dermal Studies

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 31.25, 125, or 500 mg dichloroacetic acid/kg body weight in 3.3 mL water:acetone/kg body weight 5 days per week for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous mice were administered the same doses for 39 weeks. Vehicle control mice were administered water:acetone only. Doses were applied to the clipped dorsal skin from the mid-back to the interscapular area.

Drinking Water Studies

Groups of 15 male and 15 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to 0, 500, 1,000, or 2,000 mg dichloroacetic acid/L drinking water for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to the same concentrations for 41 weeks.

Positive Control Mice

For each route of administration, positive control groups of 15 male and 15 female Tg.AC hemizygous mice were administered 1.25 µg TPA in 100 µL acetone (12.5 µg TPA/L solution) three times per week for 26 weeks. The TPA solution was applied to the clipped dorsal skin from the mid-back to the interscapular area. Positive control mice were removed from study after the appearance of 20 or more skin papillomas and discarded.

Source and Specification of Animals

Male and female FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous and B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 26-, 39- and 41-week studies. Tg.AC hemizygous mice were quarantined for 11 (drinking water) or 14 (dermal) days, and p53 haploinsufficient mice were quarantined for 12 days before the beginning of the studies. Five male and five female mice per strain were randomly selected for parasite evaluation and gross observation of disease. Tg.AC hemizygous mice were approximately 6 (dermal) or 7 to 8 (drinking water) weeks old, and p53 haploinsufficient mice were 6 to 7 weeks old at the beginning of the studies. Blood samples were collected from five male and five

female sentinel mice from each study at 4 and 26 weeks, from five male and five female mice from the highest-surviving groups from each study at 39 or 41 weeks, and from designated mice on June 9, 2000. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al*., 1986; Rao *et al*., 1989a,b). All results were negative. Animals were housed individually. Feed and water were available *ad libitum*. Water consumption was measured weekly by cage during the drinking water studies. Cages and racks were rotated every 2 weeks. Further details of the animal maintenance are summarized in Table 1.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings and body weights were recorded initially, weekly, and at the end of the studies. Clinical findings for dermal study mice were recorded postdosing.

In-life observations of papilloma formation on the skin were recorded weekly using the Toxicology Data Management System (TDMS). A papilloma was initially recorded as a mass. The observation "papilloma" was not entered into TDMS for a given animal until the first-observed mass was documented for 3 consecutive weeks. At the third observation, a mass wart-like in appearance was entered as a papilloma. Any new mass(es) appearing after the 3-week confirmation period for a given animal at a different site was entered into TDMS first as a mass until the third week, when it was entered as a papilloma. In a few instances, a papilloma that had been previously observed was missing, and therefore not recorded. Reappearance of a mass at a later time was entered into TDMS as a mass until the third observation week, when it was called a papilloma.

At the end of the 26-week studies, blood for hematology analysis was collected from the retroorbital sinus of all mice (except positive controls) under carbon dioxide anesthesia. Samples for hematology analysis were placed in microcollection tubes (Sarstedt, Inc., Nümbrecht, Germany) coated with potassium EDTA. Hematocrit; erythrocyte, platelet, and leukocyte counts; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Cell-Dyn® hematology analyzer (Abbott Diagnostics, Santa Clara, CA). Hemoglobin concentrations were determined photometrically using a cyanmethemoglobin procedure. Differential leukocyte

counts were determined microscopically from blood smears stained with a modified Wright-Giemsa stain. A Miller Disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. Mean cell volumes were determined from average red blood cell impedance pulse heights. The parameters measured are listed in Table 1.

Necropsies and microscopic evaluations were performed on all animals except the positive control groups. The heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin for microscopic evaluation. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. The quality assessment pathologist examined all tumors and all slides from potential target organs, which included the kidney and liver of Tg.AC hemizygous mice and p53 haploinsufficient mice and the skin of Tg.AC hemizygous mice in the dermal studies.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for

review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

The 26-week studies had not undergone a quality assessment review prior to completion of the pathology review for the 39- and 41-week studies. For the 26-week studies, a quality assessment pathologist evaluated all tumor diagnoses from all animals and all potential target organs (both genders, both strains, all routes of administration) which included the liver, and skin, using terminology and diagnostic criteria defined by the Pathology Working Group for the 39- and 41-week studies in order to maintain diagnostic consistency between the studies. The quality assessment pathologist and 2 NTP pathologists met to review selected examples of lesions related to chemical administration, and to address any disagreements in the diagnoses made by the laboratory and quality assessment pathologists. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, the quality assessment pathologist, and the NTP pathologists.

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Drinking Water Studies of Dichloroacetic Acid

Dermal Studies	Drinking Water Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mice	FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mice B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice
Animal Source	
Taconic Laboratory Animals and Services, Inc. (Germantown, NY)	Taconic Laboratory Animals and Services, Inc. (Germantown, NY)
Time Held Before Studies	
14 days	Tg.AC mice: 11 days p53 mice: 12 days
Average Age When Studies Began	
6 weeks	Tg.AC mice: 6 weeks p53 mice: 7 to 8 weeks (males) 6 to 7 weeks (females)
Date of First Dose or Exposure	
February 17, 2000	Tg.AC mice: January 17, 2000 p53 mice: January 18, 2000
Duration of Dosing or Exposure	
26 or 39 weeks	26 or 41 weeks
Date of Last Dose or Exposure	
August 15, 2000 (26-week study, males) August 16, 2000 (26-week study, females) November 15, 2000 (39-week study, males and females)	Tg.AC mice: July 10, 2000 (26-week study, males) July 11, 2000 (26-week study, females) October 23, 2000 (41-week study, males) October 24, 2000 (41-week study, females) p53 mice: July 12, 2000 (26-week study, males) July 13, 2000 (26-week study, females) October 25, 2000 (41-week study, males) October 26, 2000 (41-week study, females)
Necropsy Dates	
August 16, 2000 (26-week study, males) August 17, 2000 (26-week study, females) November 16, 2000 (39-week study, males and females)	Tg.AC mice: July 10, 2000 (26-week study, males) July 11, 2000 (26-week study, females) October 23, 2000 (41-week study, males) October 24, 2000 (41-week study, females) p53 mice: July 12, 2000 (26-week study, males) July 13, 2000 (26-week study, females) October 25, 2000 (41-week study, males) October 26, 2000 (41-week study, females)
Average Age at Necropsy	
32 weeks (26-week study) 45 weeks (39-week study)	Tg.AC mice: 31 weeks (26-week study) 46 weeks (26-week study) p53 mice: 30 to 32 weeks (26-week study, males) 31 weeks (26-week study, females) 45 to 47 weeks (41-week study, males) 46 weeks (41-week study, females)

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Drinking Water Studies of Dichloroacetic Acid

Dermal Studies	Drinking Water Studies
Size of Study Groups 26-week study: 15 males, 15 females 39-week study: 10 males, 10 females	26-week study: 15 males, 15 females 41-week study: 10 males, 10 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as dermal studies
Animals per Cage 1	1
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as dermal studies
Water Tap water (City of Columbus Municipal Supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Tap water (City of Columbus Municipal Supply) via amber glass bottles (Supelco, Bellefonte, PA) with stainless steel double-ball bearing sipper tubes (Ancare, Bellmore, NY) with Teflon®-lined septa, available *ad libitum*; changed weekly
Cages Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly	Same as dermal studies
Bedding Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Same as dermal studies
Rack Filters DuPont® spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as dermal studies
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as dermal studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour
Doses or Exposure Concentrations 0, 31.25, 125, or 500 mg/kg dichloroacetic acid 5 days per week or 1.25 µg TPA three times per week	0, 500, 1,000, or 2,000 mg/L dichloroacetic acid or 1.25 µg TPA three times per week
Type and Frequency of Observation Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Clinical findings were recorded postdosing.	Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly, and at the end of the studies. Water consumption was recorded weekly.

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Drinking Water Studies of Dichloroacetic Acid

Dermal Studies	Drinking Water Studies
Method of Sacrifice	
Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy	
Necropsies were performed on all mice (except positive controls). Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus	Necropsies were performed on all mice (except positive controls). Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus
Clinical Pathology	
Blood was collected from the retroorbital sinus of all mice (except positive controls) at the end of the 26-week studies for hematology. *Hematology*: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials	Same as dermal studies
Histopathology	
Histopathology was performed on all mice except positive controls. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, large intestine (cecum, colon), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pituitary gland, skin, skin (site of application), spleen, stomach (forestomach), testis (with epididymis), thymus, thyroid gland, and uterus.	Histopathology was performed on all mice except positive controls. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, large intestine (cecum, colon), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pituitary gland, spleen, stomach (forestomach), testis (with epididymis), thymus, thyroid gland, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation and Analysis of Lesion Incidences

The incidences of neoplasms or nonneoplastic lesions are presented in Appendixes A, B, and C as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

The weekly in-life skin papilloma counts were evaluated by the method of Dunson *et al*. (2000). The model separates effects on papilloma latency and multiplicity and accommodates important features of the data, including animal-to-animal variability in the expression of the transgene as reflected in the initial tumor counts. The two key parameters are γ_1, which measures the dose effect on incidence (number of animals with one or more papillomas during the study), and γ_2, which measures the dose effect on multiplicity (rate of appearance of additional papillomas after the initial papilloma has occurred). The model assumes that the rate (number of additional papillomas per time period) is exponentially increasing with respect to dose and that the rate remains constant across time.

More specifically, under the model, the increase in papilloma burden from one week to the next is assumed to be distributed as a Poisson random variable. The Poisson mean is assumed to depend on an animal-specific susceptibility variable, on exposure length, and on the dose. The rate of initial papilloma occurrence is assumed to

be log-linear in time. The coefficients for time are levels of dose multiplied by γ_1 and the animal-specific susceptibility parameters. This implies that as the dose/time increases, the rate of occurrence for the first papilloma will increase exponentially relative to increases in dose/time. A value of zero for γ_1 implies that dose is not associated with incidence (or, equivalently, the length of the latency period prior to initial onset), leaving only animal-specific characteristics to explain any variability.

After the latency period (after the first papilloma occurs), the Poisson mean changes to a rate that is only dependent on dose (that is, no animal-specific rates or dependency with time). More explicitly, the rate of occurrence of additional papillomas is assumed to be log-linear in time. A value of zero for γ_2 implies that dose is not associated with rate of additional papilloma occurrence. A non-zero value implies that the rate of additional papillomas increases with dose in a proportional fashion.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed or exposed groups and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The 26-, 39-, and 41-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Report.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1992). Dichloroacetic acid was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of dichloroacetic acid. The high dose was limited by toxicity. All positive trials were repeated under the conditions that elicited the positive response.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in

revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Mouse Peripheral Blood Micronucleus Test Protocol

Detailed discussions of this assay are presented by MacGregor *et al.* (1990) and Witt *et al.* (2000). At the end of the 26-week studies, peripheral blood samples were obtained from male and female Tg.AC hemizygous and p53 haploinsufficient mice. In addition, in another study, peripheral blood samples were obtained from B6C3F_1 mice exposed to dichloroacetic acid in drinking water (67 to 1,000 mg/L) for 3 months. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in up to 15 Tg.AC hemizygous or p53 haploinsufficient mice or 10 B6C3F_1 mice per dose or exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within an exposure group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose or exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed or exposed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed or exposed group is less than or equal to 0.025 divided by the number of dosed or exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after

considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects. Because these studies were not repeated, the results of the micronucleus trials were accepted without replication.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary tables in the Abstract of this Report present a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

26-Week Dermal Study in Tg.AC Hemizygous Mice

Positive Control Tg.AC Hemizygous Mice

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (1.25 µg) was dermally administered to groups of 15 males and 15 females three times weekly. Ninety-three percent of males and all females developed more than 20 skin papillomas each by week 18 (males) or 19 (females) (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.*, 2001).

Survival

Estimates of 26-week survival probabilities for male and female mice are shown in Table 2. Survival of dosed males and females was similar to that of the vehicle control groups.

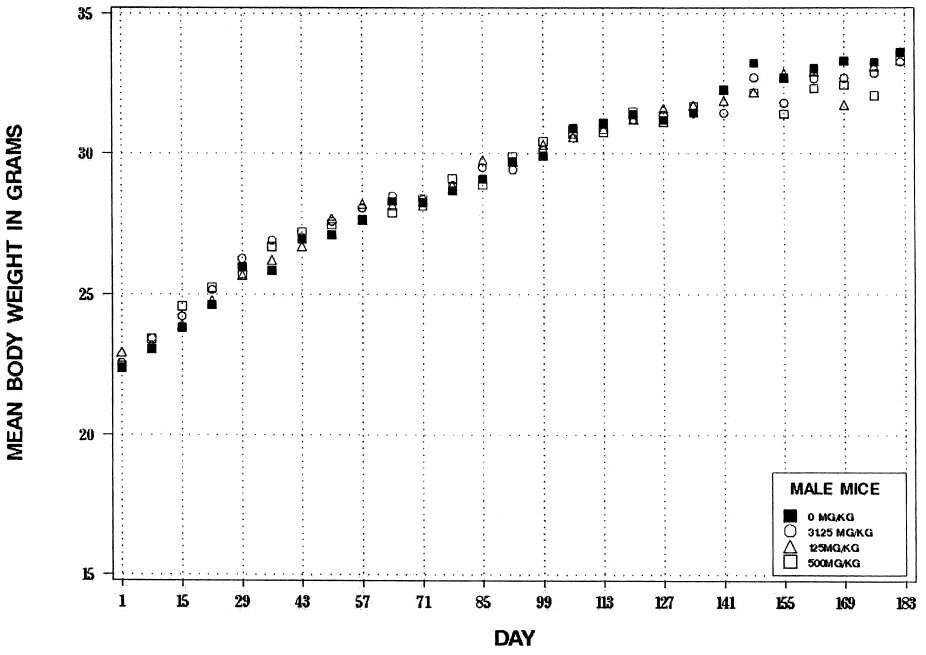
TABLE 2
Survival of Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
Animals initially in study	15	15	15	15
Moribund	2	0	0	2
Natural deaths	0	1	1	1
Animals surviving to study termination	13	14	14	12
Percent probability of survival at end of study[a]	87	93	93	80
Mean survival (days)[b]	176	180	175	156
Survival analysis[c]	P=0.455	P=0.984N	P=1.000N	P=0.922
Female				
Animals initially in study	15	15	15	15
Moribund	0	1	0	0
Natural deaths	4	2	1	0
Animals surviving to study termination	11	12	14	15
Percent probability of survival at end of study	73	80	93	100
Mean survival (days)	159	170	177	183
Survival analysis	P=0.088N	P=0.922N	P=0.320N	P=0.107N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dosed group is indicated by **N**.

Body Weights and Clinical Findings

The mean body weights of all dosed groups of males were similar to those of the vehicle control group throughout the study; mean body weights of 31.25, 125, and 500 mg/kg females were greater than those of the vehicle control group after weeks 22, 19, and 21, respectively (Figure 1 and Tables 3 and 4). Clinical findings included papillomas, primarily on the head and in the genital area, in males (5/15, 0/15, 3/15, 6/15) and did not appear to be related to administration of dichloroacetic acid (data not shown). The in-life observations of the papillomas at the site of application were combined with the 39-week study and are shown in Table 10, which appears in the 39-week section of these results.



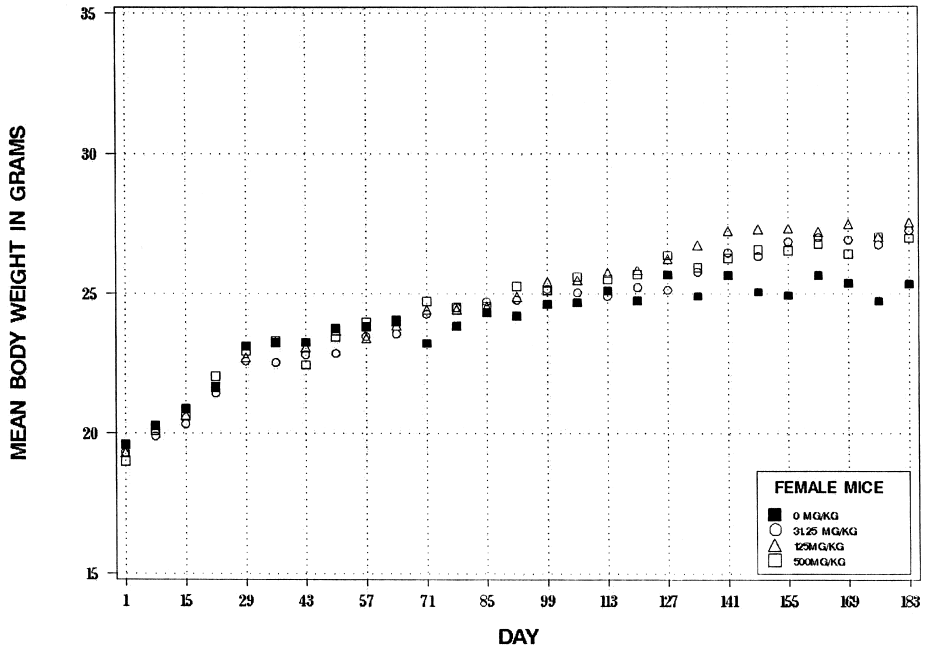


FIGURE 1
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Dichloroacetic Acid Dermally for 26 Weeks

TABLE 3
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

Weeks on Study	Vehicle Control		31.25 mg/kg			125 mg/kg			500 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.4	15	22.6	101	15	22.9	102	15	22.5	100	15
2	23.0	15	23.4	102	15	23.2	101	15	23.4	102	14
3	23.8	15	24.2	102	15	23.9	100	15	24.6	103	14
4	24.6	15	25.1	102	15	24.8	101	15	25.2	102	14
5	26.0	15	26.3	101	15	25.7	99	15	25.7	99	14
6	25.8	15	26.9	104	15	26.2	102	15	26.7	104	14
7	27.0	15	27.0	100	15	26.7	99	15	27.2	101	14
8	27.1	15	27.6	102	15	27.7	102	15	27.5	102	14
9	27.7	15	28.1	101	15	28.2	102	15	27.6	100	14
10	28.3	15	28.5	101	15	28.1	99	15	27.9	99	14
11	28.3	15	28.4	100	15	28.1	99	15	28.4	100	14
12	28.7	15	28.9	101	15	28.9	101	15	29.1	101	13
13	29.1	15	29.5	101	15	29.8	102	14	28.9	99	13
14	29.7	15	29.4	99	15	29.7	100	14	29.9	101	13
15	29.9	15	30.1	101	15	30.3	101	14	30.4	102	13
16	30.9	15	30.6	99	15	30.6	99	14	30.7	99	13
17	31.1	15	31.1	100	15	30.9	99	14	30.8	99	13
18	31.4	14	31.2	99	15	31.2	99	14	31.5	100	13
19	31.2	14	31.4	101	15	31.6	101	14	31.1	100	13
20	31.5	14	31.4	98	15	31.7	101	14	31.7	101	12
21	32.3	14	31.4	97	15	31.9	99	14	32.3	100	12
22	33.2	14	32.7	99	14	32.2	97	14	32.2	97	12
23	32.7	13	31.8	97	14	32.9	101	14	31.4	96	12
24	33.0	13	32.7	99	14	32.9	100	14	32.3	98	12
25	33.3	13	32.7	98	14	31.7	95	14	32.4	97	12
26	33.3	13	32.9	99	14	33.1	99	14	32.1	97	12
Mean for weeks											
1-13	26.3		26.7	102		26.5	101		26.5	101	
14-26	31.8		31.5	99		31.6	99		31.4	99	

TABLE 4
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

Weeks on Study	Vehicle Control		31.25 mg/kg			125 mg/kg			500 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.6	15	19.3	99	15	19.3	99	15	19.0	97	15
2	20.3	15	19.9	98	15	20.2	100	15	20.1	99	15
3	20.9	15	20.3	97	15	20.6	99	15	20.6	99	15
4	21.7	15	21.4	99	15	21.6	100	15	22.0	101	15
5	23.1	15	22.6	98	15	22.7	98	15	22.9	99	15
6	23.3	15	22.5	97	15	23.2	100	15	23.3	100	15
7	23.3	15	22.8	98	15	23.1	99	15	22.4	96	15
8	23.7	15	22.8	96	15	23.7	100	15	23.4	99	15
9	23.8	14	23.5	99	15	23.4	98	15	24.0	101	15
10	24.1	14	23.6	98	15	23.9	99	15	24.0	100	15
11	23.2	13	24.3	105	15	24.4	105	15	24.7	107	15
12	23.8	13	24.5	103	15	24.4	103	15	24.5	103	15
13	24.3	13	24.7	102	15	24.5	101	15	24.6	101	15
14	24.2	13	24.8	103	15	24.9	103	15	25.3	105	15
15	24.6	13	25.2	102	14	25.4	103	14	25.1	102	15
16	24.7	12	25.0	101	14	25.5	103	14	25.6	104	15
17	25.1	12	24.9	99	14	25.8	103	14	25.5	102	15
18	24.8	12	25.2	102	14	25.8	104	14	25.7	104	15
19	25.7	12	25.1	98	14	26.2	102	14	26.4	103	15
20	24.9	12	25.8	104	12	26.7	107	14	25.9	104	15
21	25.7	12	26.5	103	12	27.2	106	14	26.3	102	15
22	25.1	11	26.3	105	12	27.3	109	14	26.6	106	15
23	24.9	11	26.8	108	12	27.3	110	14	26.5	106	15
24	25.6	11	27.0	106	12	27.2	106	14	26.8	105	15
25	25.4	11	26.9	106	12	27.5	108	14	26.4	104	15
26	24.7	11	26.7	108	12	27.0	109	14	27.0	109	15
Mean for weeks											
1-13	22.7		22.5	99		22.7	100		22.7	100	
14-26	25.0		25.9	104		26.5	106		26.1	104	

Hematology

Hematology data are shown in Table E1. A minimal decrease (2%) in mean cell hemoglobin concentration was statistically identified in the 500 mg/kg males, and a minimal increase (approximately 8%) in platelet counts occurred in the 125 and 500 mg/kg males. The value, in both cases, was not outside what would be considered an acceptable reference limit and was not considered clinically or toxicologically relevant.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the skin, forestomach, liver, and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables A1 through A4.

Skin: Squamous cell papillomas occurred at the site of application in one 125 mg/kg male, in two 500 mg/kg males, and in two 500 mg/kg females (Tables 5, A1, and A3). In both males and females, the number of mice bearing papillomas, the time to first appearance, the total number of papillomas, and the mean number of papillomas per mouse (for surviving mice with papillomas) were similar to vehicle controls in the 31.25 and 125 mg/kg groups regardless of the site of the papilloma. Each of these parameters was slightly increased relative to vehicle controls in the 500 mg/kg group. At the site of application, the incidences of epidermal hyperplasia and epidermal hyperkeratosis in the 125 and 500 mg/kg male and female groups were significantly increased. Hyperplasia of the epidermis was characterized by focal thickening of the stratified squamous epithelium. An associated hyperkeratosis was usually present. Squamous cell papillomas of the skin were usually characterized by frond-like projections that radiated from a fibrovascular stalk. However, some papillomas lacked the typical frond-like projections.

TABLE 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin in Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
Number Examined Microscopically	15	15	15	15
Epidermis, Hyperplasia[a]	2 (2.5)[b]	3 (3.3)	2 (2.0)	3 (1.7)
Site of Application, Epidermis, Hyperkeratosis	2 (1.5)	7 (1.1)	15** (1.9)	14** (2.0)
Site of Application, Epidermis, Hyperplasia	0	2 (1.0)	11** (1.0)	13** (1.8)
Squamous Cell Papilloma, Multiple	0	2	1	2
Squamous Cell Papilloma (includes multiple)	5	5	3	5
Site of Application, Squamous Cell Papilloma, Multiple	0	0	0	1
Site of Application, Squamous Cell Papilloma (includes multiple)	0	0	1	2
Female				
Number Examined Microscopically	15	15	15	15
Hyperkeratosis	1 (3.0)	0	1 (3.0)	0
Epidermis, Hyperplasia	3 (2.7)	1 (2.0)	1 (4.0)	2 (2.5)
Site of Application, Epidermis, Hyperkeratosis	8 (1.3)	9 (1.0)	14* (1.6)	14* (1.9)
Site of Application, Epidermis, Hyperplasia	0	1 (1.0)	10** (1.0)	13** (1.8)
Squamous Cell Papilloma, Multiple	0	3	2	0
Squamous Cell Papilloma (includes multiple)	6	8	8	2
Site of Application, Squamous Cell Papilloma	0	0	0	2

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Forestomach: The incidences of forestomach squamous cell papilloma were increased in dosed males, especially at 31.25 mg/kg (1/15, 6/15, 3/15, 3/15; Table A1). There were no increases in forestomach papillomas in females (7/15, 6/15, 8/15, 7/15; Table A3). The increase in males was not considered related to treatment because it was not dose-related, there were no increased incidences in males at 39 weeks (7/10, 6/10, 8/10, 7/10; Table A5), and the concurrent 26-week control rate appeared low (in the companion 26-week drinking water study, 4/15 control males had forestomach papillomas; Table B1). In addition, the incidences of forestomach papilloma were not increased in 26- or 39-week females (Tables A3 and A7).

Liver: The absolute and relative liver weights of 125 and 500 mg/kg males and 500 mg/kg females were significantly greater than those of the vehicle controls (Table F1). The incidences of hepatocyte cytoplasmic vacuolization were significantly increased in males and females in the 125 and 500 mg/kg groups, and the average severity generally increased with increasing dose (Tables 6 and A2, and A4). Hepatocyte cytoplasmic vacuolization was characterized by poorly demarcated cytoplasmic clear spaces that lacked distinct borders and that were partially separated by irregular strands of eosinophilic cytoplasm. This change was considered consistent with cytoplasmic glycogen accumulation. The vacuole area sometimes displayed light basophilic stippling, a discoloration that was only occasionally observed in controls but more frequent in the treated animals. The pattern of accumulation tended to be centrilobular but as severity increased involved more of the hepatocytes within the lobules. Hepatocyte vacuolization was graded according to the following criteria: minimal vacuolization was slight and consisted of small poorly demarcated, usually perinuclear, clear spaces in the cytoplasm that were barely visible at low magnification. Mild vacuolization tended to be more prominent at low magnification; vacuoles were irregular and coalesced but did not distend the cytoplasm and were not associated with increase in cell size. Moderate vacuolization was characterized by vacuoles that clearly coalesced and the hepatocyte cytoplasm was expanded one and a half to twice the normal cell size compared to that of the controls. Hepatocyte nuclei were typically centrally located. Marked hepatocyte vacuolization consisted of an exaggeration of the features described above with an increase in hepatocyte size that was approximately two times or more normal size. Hepatocyte vacuolization was frequently accompanied by the presence of discrete clear cytoplasmic vacuoles consistent with cytoplasmic lipid accumulation and diagnosed as fatty change.

Kidney: The incidence of nephropathy was significantly increased in the 500 mg/kg males; however, the severity was similar to that of the vehicle controls (Tables 6 and A2). Nephropathy consisted of a spectrum of changes that occurred alone or in combination and included small clusters of tubules with cytoplasmic basophilia (regeneration), tubular dilatation and proteinaceous casts, and interstitial mononuclear inflammatory cell infiltration.

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
Liver[a]	15	15	15	15
Hepatocyte, Vacuolization Cytoplasmic[b]	3 (1.0)[c]	4 (1.0)	14** (1.8)	15** (2.8)
Kidney	15	15	15	15
Nephropathy	7 (1.0)	7 (1.0)	11 (1.0)	13* (1.0)
Female				
Liver	15	15	15	15
Hepatocyte, Vacuolization Cytoplasmic	6 (1.2)	4 (1.0)	14** (2.1)	15** (3.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

39-Week Dermal Study in Tg.AC Hemizygous Mice

Survival

Estimates of 39-week survival probabilities for male and female mice are shown in Table 7. Survival of dosed males and females was similar to that of the vehicle controls.

Table 7
Survival of Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
Animals initially in study	10	10	10	10
Moribund	1	3	1	2
Natural deaths	0	1	1	1
Animals surviving to study termination	9	6	8	7
Percent probability of survival at end of study[a]	90	60	80	70
Mean survival (days)[b]	268	236	263	256
Survival analysis[c]	P=0.980	P=0.275	P=1.000	P=0.578
Female				
Animals initially in study	10	10	10	10
Moribund	2	3	2	2
Natural deaths	0	2	2	0
Animals surviving to study termination	8	5	6	8
Percent probability of survival at end of study	80	50	60	80
Mean survival (days)	246	222	251	249
Survival analysis	P=0.618N	P=0.356	P=0.679	P=1.000N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dosed group is indicated by **N**.

Body Weights and Clinical Findings

The mean body weights of the 31.25 mg/kg males were less than those of the vehicle controls after week 22, and those of 500 mg/kg males were less than those of the vehicle controls after week 21; although mean body weights of 125 mg/kg males were less from weeks 28 to 38, the mean body weight was similar to that of the vehicle controls at the end of the study (Table 8 and Figure 2). The mean body weights of 500 mg/kg females were greater than those of the vehicle controls after week 17 and the mean body weights of the 31.25 and 125 mg/kg groups were greater at the end of the study (Table 9 and Figure 2).

TABLE 8
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

Weeks on Study	Vehicle Control		31.25 mg/kg			125 mg/kg			500 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.9	10	22.7	99	10	22.6	99	10	22.7	99	10
2	23.1	10	23.4	101	10	23.4	101	10	23.5	102	10
3	24.2	10	23.8	98	10	24.4	101	10	24.1	100	10
4	25.2	10	24.7	98	10	25.0	99	10	25.1	100	10
5	25.8	10	25.0	97	10	25.5	99	10	25.9	100	10
6	27.1	10	26.2	97	10	26.3	97	10	26.2	97	10
7	27.0	10	25.9	96	10	26.3	97	10	27.1	100	10
8	28.5	10	27.4	96	10	27.2	95	10	27.4	96	10
9	29.1	10	27.6	95	10	28.1	97	10	27.6	95	10
10	29.2	10	27.8	95	10	28.0	96	10	28.2	97	10
11	29.2	10	28.5	98	10	28.8	99	10	28.6	98	10
12	29.8	10	28.3	95	10	28.9	97	10	28.9	97	10
13	30.8	10	29.2	95	10	29.7	96	10	29.5	96	10
14	30.8	10	29.3	95	10	29.5	96	10	29.7	96	10
15	31.3	10	30.2	97	10	30.7	98	10	30.1	96	10
16	31.9	10	29.9	94	10	29.6	93	10	30.4	95	10
17	31.5	10	30.9	98	10	31.1	99	10	30.4	97	10
18	32.4	10	30.7	95	10	31.7	98	10	30.6	94	10
19	32.2	10	30.9	96	9	31.2	97	10	31.6	98	10
20	33.0	10	31.7	96	9	31.9	97	10	31.8	96	10
21	33.3	10	31.6	95	9	33.2	100	10	32.0	96	10
22	33.3	10	31.9	96	9	33.1	99	10	31.3	94	10
23	34.7	10	32.2	93	9	32.5	94	10	31.6	91	10
24	34.4	10	32.1	93	8	33.4	97	10	32.2	94	10
25	33.9	10	31.4	93	8	33.2	98	10	32.0	94	10
26	34.0	10	32.4	95	8	34.0	100	10	32.1	94	9
27	34.5	10	32.4	94	8	32.6	95	10	31.5	91	9
28	35.3	10	31.9	90	8	32.1	91	10	32.2	91	9
29	36.4	10	32.2	89	8	33.4	92	10	32.2	89	9
30	36.2	10	31.1	86	8	33.4	92	10	32.6	90	9
31	36.6	10	31.5	86	8	33.0	90	10	32.2	88	9
32	37.2	9	32.6	88	7	34.2	92	9	33.0	89	9
33	38.2	9	33.0	86	7	34.3	90	9	32.3	85	9
34	38.2	9	33.8	89	6	35.0	92	8	34.2	90	8
35	38.3	9	33.3	87	6	34.9	91	8	33.6	88	8
36	39.1	9	33.6	86	6	34.1	87	8	33.8	86	7
37	38.5	9	34.1	89	6	35.8	93	8	34.2	89	7
38	38.2	9	33.8	89	6	36.0	94	8	34.1	89	7
39	37.4	9	33.9	91	6	36.5	98	8	34.3	92	7
Mean for weeks											
1-13	27.1		26.2	97		26.5	98		26.5	98	
14-39	35.0		32.0	91		33.1	95		32.2	92	

TABLE 9
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

Weeks on Study	Vehicle Control		31.25 mg/kg			125 mg/kg			500 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.5	0	19.9	102	10	19.7	101	10	19.7	101	10
2	19.8	10	19.8	100	10	19.9	101	10	20.2	102	10
3	19.8	10	20.5	104	10	20.9	106	10	20.2	102	10
4	21.7	10	21.6	100	9	22.0	101	10	22.0	101	10
5	22.8	10	20.0	88	9	22.4	98	10	21.9	96	10
6	23.1	10	22.7	98	9	23.1	100	10	23.4	101	10
7	23.1	10	22.4	97	9	22.9	99	10	22.1	96	10
8	23.2	10	22.8	98	9	23.2	100	10	20.9	90	10
9	23.3	10	23.1	99	9	23.6	101	10	23.7	102	9
10	24.0	10	23.1	96	9	23.5	98	10	23.8	99	9
11	23.9	10	23.5	98	9	24.1	101	9	24.0	100	9
12	24.4	10	24.2	99	9	25.1	103	9	24.7	101	9
13	24.7	10	24.3	98	9	24.8	100	9	25.2	102	9
14	24.6	10	24.1	98	9	25.0	102	9	25.4	103	9
15	25.4	10	24.2	95	9	25.2	99	9	25.7	101	9
16	24.8	10	24.5	99	9	25.2	102	9	26.0	105	9
17	25.5	10	24.3	95	8	25.6	100	9	26.1	102	9
18	25.3	9	24.7	98	8	26.0	103	9	26.7	106	9
19	25.1	9	24.7	98	8	25.0	100	9	26.7	106	9
20	24.9	9	25.3	102	8	25.0	100	9	26.9	108	9
21	25.0	9	25.7	103	8	26.7	107	9	27.6	110	9
22	25.9	8	25.4	98	8	26.5	102	9	28.7	111	9
23	26.3	8	25.7	98	8	26.8	102	9	29.0	110	9
24	26.2	8	25.8	99	8	27.1	103	9	29.4	112	9
25	26.7	8	25.7	96	8	26.9	101	9	29.6	111	9
26	26.9	8	26.0	97	8	27.0	100	9	29.3	109	9
27	26.6	8	26.4	99	8	27.1	102	9	30.5	115	9
28	27.2	8	26.4	97	8	27.2	100	9	31.0	114	9
29	27.3	8	27.1	99	8	27.3	100	9	31.6	116	9
30	28.0	8	26.7	95	8	27.9	100	9	31.0	111	9
31	27.6	8	26.6	96	8	28.0	101	9	31.2	113	9
32	27.5	8	27.3	99	7	28.0	102	9	31.4	114	9
33	27.5	8	27.7	101	7	28.6	104	9	32.0	116	9
34	27.0	8	27.0	100	7	27.9	103	9	32.6	121	9
35	26.5	8	27.1	102	7	28.3	107	9	32.2	122	9
36	27.1	8	26.3	97	7	28.6	106	9	32.6	120	9
37	27.4	8	27.3	100	6	28.4	104	9	34.5	126	8
38	27.2	8	28.0	103	5	27.3	100	9	33.3	122	8
39	25.7	8	27.8	108	5	29.9	116	7	35.3	137	8
Mean for weeks											
1-13	22.6		22.1	98		22.7	100		22.4	99	
14-39	26.4		26.1	99		27.0	102		29.9	113	

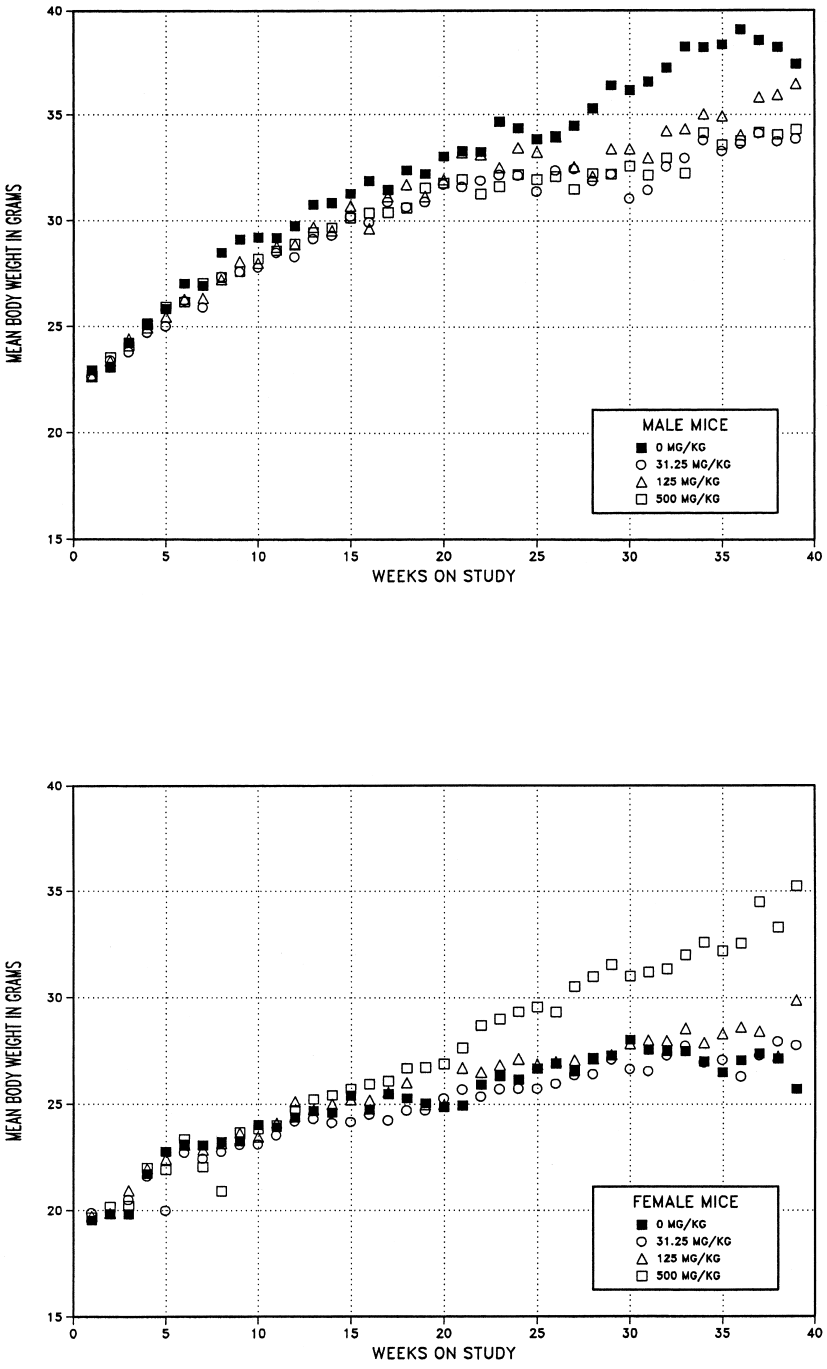


FIGURE 2
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Dichloroacetic Acid Dermally for 39 Weeks

Chemical-related clinical findings included observations of papillomas at the site of application in 125 and 500 mg/kg males (vehicle control, 0/10; 31.25 mg/kg, 0/10; 125 mg/kg, 2/10; 500 mg/kg, 8/10) and females (0/10, 0/10, 1/10, 5/10). While vehicle control animals did not have papillomas at the site of application, papillomas were observed in these groups at nonapplication sites (males: 5/10, 0/10, 3/10, 6/10; females: 5/10, 8/10, 5/10, 3/10).

Because the 26- and 39-week studies were conducted concurrently, papilloma formation data were combined and analyzed using the model of Dunson *et al*. (2000). For males and females, there were significant dose-related trends in time to first papilloma and in the number of papillomas per animal (Table 10). Furthermore, the time to first tumor in the 500 mg/kg group was significantly less than in the control group for males and females.

TABLE 10
Skin Papilloma Formation at the Site of Application in Tg.AC Hemizygous Mice in the 26- and 39-Week Dermal Studies of Dichloroacetic Acid[a]

Dose (mg/kg)	Number (Percent) with Papilloma[b]	Time to Initial Papilloma Occurrence for All Animals in Group[c] (Week)		Distribution of Number of Papillomas per Animal[d] Quantiles			Test of Dunson *et al.* Model[e]	
		First	Median	10th	50th	90th	γ_1	γ_2
Male								
0	0 (0.0%)	>39	>39	0	0	0		
31.25	1 (4.0%)	18	>39	0	0	0	NT	NT
125	1 (4.0%)	28	>39	0	0	0	NT	NT
500	10 (40.0%)	16	>39	0	0	4	*	NT
Trend							*	*
Positive Control[f]	14 (99.8%)	9	11	20+	20+	20+	*	NT
Female								
0	0 (0.0%)	>39	>39	0	0	0		
31.25	0 (0.0%)	>39	>39	0	0	0	NT	NT
125	2 (8.0%)	27	>39	0	0	0	NT	NT
500	10 (40.0%)	16	>39	0	0	2	*	NT
Trend							*	*
Positive Control	15 (100.0%)	9	11	20+	20+	20+	*	NT

[a] 25 males and 25 females initially in each dose group; 15 males and 15 females initially in positive control group
[b] Percent is Poly-3 adjusted rate and reflects whether the animal ever had a confirmed papilloma at any point in the study.
[c] If the first papilloma was observed at the terminal sacrifice, it was assigned a time to first occurrence of the sacrifice time. For groups in which fewer than half of the animals had papillomas, the median time to initial occurrence is >39 weeks.
[d] Quantiles are based on all animals in a group, whether removed before the end of study or not. For example, a value of 4 for the 90th quantile implies that 90% of the animals in the study had 4 papillomas or fewer at the end of the study (or at removal from study).
[e] The Dunson *et al.* (2000) model accounts for latency (γ_1) and multiplicity (γ_2) in the rate of occurrence, NT (No Test) indicates that these data do not support a pairwise test. * ($P \leq 0.05$) indicates a significant trend or a significant difference from the vehicle control group.
[f] 100 µL of 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate per 100 mL acetone administered three times per week.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the skin, liver, kidney, thymus, and testes. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables A5 through A8.

Skin: At the site of application, the incidences of epidermal hyperplasia were significantly increased in the 125 and 500 mg/kg males and the 500 mg/kg females (Tables 11, A6, and A8). Epidermal hyperkeratosis occurred in all groups of males and females, with significantly increased incidences in 31.25 and 125 mg/kg males and 500 mg/kg males and females. The incidences of squamous cell papillomas at the site of application were significantly increased in the 500 mg/kg males and females (Tables 11, A5, and A7).

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin in Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
Number Examined Microscopically	10	10	10	10
Site of Application				
Epidermis, Hyperkeratosis[a]	2 (1.0)[b]	8* (1.3)	9** (1.6)	10** (2.1)
Epidermis, Hyperplasia	0	0	8** (1.3)	9** (2.2)
Squamous Cell Papilloma, Multiple	0	0	1	6**
Squamous Cell Papilloma (includes multiple)	0	0	2	8**
Other than Site of Application				
Squamous Cell Papilloma, Multiple	6	4	7	2
Squamous Cell Papilloma (includes multiple)	8	4	7	9
Female				
Number Examined Microscopically	10	10	10	10
Site of Application				
Epidermis, Hyperkeratosis	5 (1.4)	8 (1.0)	9 (1.4)	10* (1.0)
Epidermis, Hyperplasia	0	0	3 (1.3)	6** (1.3)
Squamous Cell Papilloma, Multiple	0	0	0	3
Squamous Cell Papilloma (includes multiple)	0	0	0	6**
Other than Site of Application				
Squamous Cell Papilloma, Multiple	5	2	4	4
Squamous Cell Papilloma (includes multiple)	7	5	5	7

* Significantly different ($P \le 0.05$) from the vehicle control group by the Fisher exact test
** $P \le 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Liver: There was a dose-related increase in the mean severity of hepatocyte cytoplasmic vacuolization in males and females; the severity increased in dosed groups from minimal at 31.25 mg/kg, mild at 125 mg/kg, to moderate at 500 mg/kg (Tables 12, A5, and A7). The incidence of hepatocyte necrosis was significantly increased in the 500 mg/kg males. Hepatocyte cytoplasmic vacuolization was morphologically similar to that previously described in the 26-week study.

Kidney: The incidences of nephropathy were significantly increased in all dosed groups of males; however, the severities of nephropathy in the dosed groups were similar to that of the vehicle controls (Tables 12 and A6). Nephropathy was morphologically similar to that previously described in the 26-week study.

Thymus: There was a significantly increased incidence of atrophy in 500 mg/kg males, but the increase was not considered dose related (Tables 12 and A6).

Testes: The incidence of germinal epithelium degeneration in 500 mg/kg males was significantly increased; however, the increased incidence was not dose related (Tables 12 and A6). Degeneration was unilateral in the 0, 31.25, and 125 mg/kg groups but bilateral in the 500 mg/kg dose group and consisted of vacuolization and loss of germinal epithelial cells, disruption of the normal tubular architecture, and occasional atrophy of affected tubules.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control		31.25 mg/kg		125 mg/kg		500 mg/kg	
Male								
Liver[a]	10		10		10		10	
Hepatocyte, Necrosis[b]	1	(1.0)[c]	0		2	(1.0)	6*	(1.0)
Hepatocyte, Vacuolization Cytoplasmic	9	(1.1)	7	(1.3)	8	(2.3)	10	(3.0)
Kidney	10		10		10		10	
Nephropathy	2	(1.0)	7*	(1.0)	7*	(1.0)	8*	(1.0)
Thymus	9		10		10		9	
Atrophy	0		3	(2.7)	1	(3.0)	4*	(2.8)
Testes	10		10		10		10	
Germinal Epithelium, Degeneration	3	(3.7)	2	(3.0)	1	(2.0)	8*	(2.3)
Female								
Liver	10		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic	7	(1.0)	6	(1.2)	8	(2.0)	10	(2.7)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

26-Week Drinking Water Study in Tg.AC Hemizygous Mice

Survival

Estimates of 26-week survival probabilities for male and female mice are shown in Table 13. Survival of all exposed groups of males was similar to that of the control group. The survival of the 500 and 2,000 mg/L females was significantly lower than that of the control group.

Table 13
Survival of Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Animals initially in study	15	15	15	15
Moribund	1	2	4	1
Animals surviving to study termination	14	13	11	14
Percent probability of survival at end of study[a]	93	87	73	93
Mean survival (days)[b]	169	161	167	173
Survival analysis[c]	P=1.000N	P=0.984	P=0.384	P=1.000N
Female				
Animals initially in study	15	15	15	15
Moribund	0	4	1	4
Natural deaths	0	3	1	1
Animals surviving to study termination	15	8	13	10
Percent probability of survival at end of study	100	53	87	67
Mean survival (days)	177	148	166	155
Survival analysis	P=0.256	P=0.009	P=0.464	P=0.050

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

Body Weights, Water and Compound Consumption, and Clinical Findings

The mean body weights of 500 mg/L males were greater than those of the controls after week 17 and those of 1,000 mg/L males were greater after week 21 (Tables 14 and 15; Figure 3). The mean body weights of 1,000 and 2,000 mg/L females were generally less than those of the controls, after weeks 16 and 15, respectively. Water consumption by 2,000 mg/L males and females was less than that by the controls throughout most of the study (Tables H1 and H2). Drinking water concentrations of 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 76, 145, and 240 mg dichloroacetic acid/kg body weight to males and 103, 180, and 298 mg/kg to females.

Hematology

Hematology data are shown in Table E2. No changes that were considered clinically or toxicologically relevant occurred.

TABLE 14
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.2	15	21.9	103	15	21.7	102	15	22.0	104	15
2	22.5	15	22.8	101	15	23.2	103	15	22.8	101	15
3	23.7	15	24.3	103	15	25.1	106	15	24.8	105	15
4	25.4	15	26.3	104	15	26.4	104	15	26.1	103	15
5	26.4	15	27.4	104	15	27.3	103	15	27.0	102	15
6	27.1	15	27.7	102	15	27.4	101	15	27.8	103	15
7	27.8	15	28.2	101	15	28.1	101	15	27.9	100	15
8	28.4	15	29.0	102	15	29.1	103	15	28.5	100	15
9	28.8	15	29.3	102	15	30.0	104	15	28.9	100	15
10	28.6	15	30.2	106	14	29.8	104	15	28.8	101	15
11	29.7	14	30.8	104	14	30.6	103	15	29.9	101	15
12	30.8	14	31.3	102	13	30.8	100	15	30.9	100	15
13	31.0	14	31.6	102	13	31.6	102	15	31.2	101	15
14	31.2	14	32.0	103	13	31.6	101	15	31.3	100	15
15	31.5	14	32.2	102	13	31.6	100	15	31.5	100	15
16	31.3	14	32.5	104	13	31.8	102	15	31.6	101	15
17	31.5	14	33.4	106	13	31.9	101	15	32.1	102	15
18	31.7	14	33.7	106	13	32.3	102	15	32.0	101	15
19	32.5	14	34.7	107	13	33.4	103	15	33.3	103	15
20	32.8	14	35.3	108	13	32.8	100	15	32.8	100	14
21	33.2	14	35.7	108	13	31.7	96	15	33.1	100	14
22	33.6	14	35.7	106	13	35.7	106	11	33.3	99	14
23	33.4	14	35.4	106	13	35.7	107	11	33.9	102	14
24	33.8	14	35.4	105	13	35.9	106	11	33.7	100	14
25	34.5	14	35.5	103	13	36.5	106	11	34.1	99	14
Mean for weeks											
1-13	27.0		27.8	103		27.8	103		27.4	101	
14-25	32.6		34.3	105		33.4	102		32.7	100	

TABLE 15
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.7	15	18.7	100	15	18.8	101	15	19.0	102	15
2	18.9	15	19.2	102	15	19.6	104	15	19.3	102	15
3	19.4	15	20.5	106	15	20.5	106	15	20.5	106	15
4	21.5	15	21.3	99	15	21.7	101	15	21.2	99	15
5	22.3	15	22.2	100	15	22.2	100	15	21.9	98	15
6	22.8	15	22.2	97	15	22.8	100	15	21.9	96	15
7	22.9	15	22.2	97	14	23.1	101	15	22.4	98	15
8	24.0	15	23.2	97	14	23.7	99	15	22.8	95	15
9	24.3	15	24.3	100	14	24.4	100	15	23.2	96	15
10	24.2	15	24.9	103	14	24.4	101	14	22.6	93	15
11	24.6	15	24.9	101	14	25.1	102	14	23.1	94	13
12	25.1	15	24.7	98	14	24.8	99	14	23.9	95	13
13	25.5	15	25.2	99	14	24.8	97	14	24.1	95	13
14	25.5	15	25.9	102	13	25.2	99	14	24.6	97	13
15	26.0	15	26.2	101	13	25.3	97	14	24.9	96	13
16	26.6	15	26.5	100	13	25.3	95	14	25.0	94	13
17	27.3	15	27.2	100	12	25.3	93	14	24.7	91	13
18	27.3	15	27.4	100	11	25.7	94	14	24.6	90	12
19	27.6	15	29.6	107	11	25.8	94	14	25.2	91	12
20	28.0	15	30.1	108	10	27.2	97	13	25.2	90	12
21	29.1	15	29.7	102	10	27.2	94	13	25.2	87	12
22	29.4	15	28.7	98	10	27.4	93	13	25.9	88	11
23	29.9	15	30.5	102	9	28.2	94	13	25.9	87	11
24	30.2	15	30.5	101	9	28.4	94	13	26.7	88	11
25	30.7	15	32.2	105	8	28.8	94	13	28.0	91	10
Mean for weeks											
1-13	22.6		22.6	100		22.8	101		22.0	97	
14-25	28.1		28.7	102		26.7	95		25.5	91	

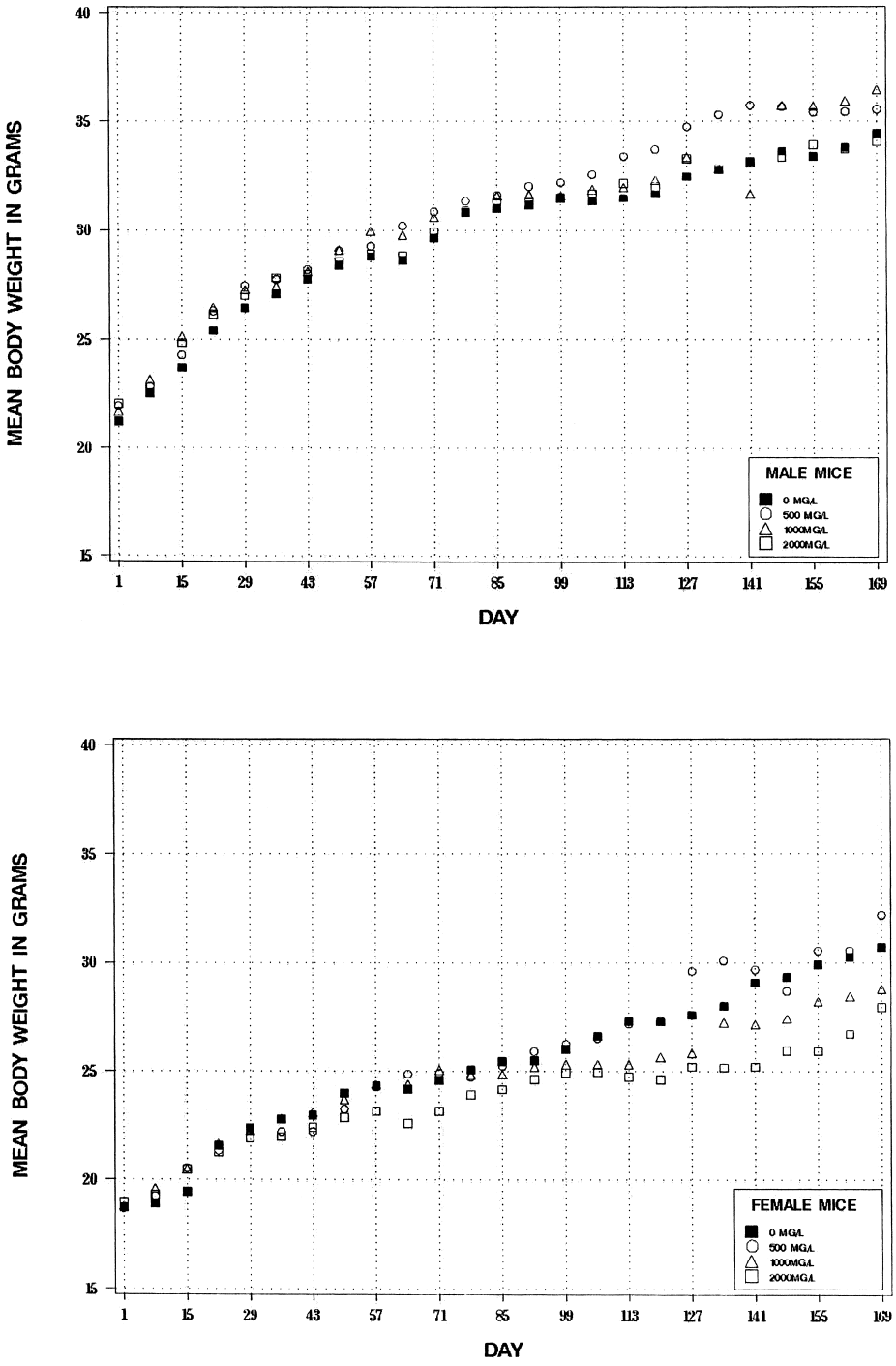


FIGURE 3
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Dichloroacetic Acid in Drinking Water for 26 Weeks

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the salivary gland, lung, liver, skin, and forestomach. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables B1 through B4.

Salivary gland: The incidences of carcinoma were slightly increased in 1,000 and 2,000 mg/L males (0 mg/L, 0/0; 500 mg/L, 0/0; 1,000 mg/L, 1/1; 2,000 mg/L, 1/1; Table B1). No adenomas were observed in this study.

Lung: An alveolar/bronchiolar carcinoma was present in one 1,000 mg/L male, one 500 mg/L female, and one 2,000 mg/L female (males: 0/15, 0/15, 1/15, 0/15; females: 0/15, 1/15, 0/15, 1/15; Tables B1 and B3).

Liver: The incidences of hepatocyte cytoplasmic vacuolization were significantly increased in all exposed groups of males and in 1,000 and 2,000 mg/L females; in both sexes, the average severity of hepatocyte cytoplasmic vacuolization increased with increasing exposure concentration (Tables 16, B2, and B4). Hepatocyte cytoplasmic vacuolization was morphologically similar to that previously described in the 26-week dermal study.

Skin: There was an increased incidence of epidermal hyperplasia in 2,000 mg/L females, but the increase was not statistically significant (Tables 16 and B4). The incidences of squamous cell papilloma in exposed groups of mice were similar to those in the controls (Tables B1 and B3). Hyperplasia was morphologically similar to that previously described in the 26-week dermal study.

Forestomach: There were slight, though not statistically significant, increases in the incidences of epithelial hyperplasia in 2,000 mg/L males and 500 mg/L females (Tables 16, B2, and B4). The incidence of epithelial hyperkeratosis was significantly increased in 500 mg/L females. The incidences of squamous cell papilloma in exposed groups of mice were similar to those in the controls (Tables B1 and B3).

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L		500 mg/L		1,000 mg/L		2,000 mg/L	
Male								
Liver[a]	15		15		15		15	
Hepatocyte, Vacuolization Cytoplasmic[b]	7	(1.0)	13*	(1.8)	15**	(2.7)	15**	(3.7)
Stomach, Forestomach	15		15		15		15	
Epithelium, Hyperkeratosis	0		0		1	(2.0)	1	(2.0)
Epithelium, Hyperplasia	2	(1.0)	4	(2.0)	3	(2.0)	6	(2.0)
Female								
Liver	15		15		15		15	
Hepatocyte, Vacuolization Cytoplasmic	6	(1.3)	10	(2.7)	14**	(3.1)	14**	(3.7)
Skin	4		4		2		8	
Epidermis, Hyperplasia	0		0		1	(3.0)	4	(2.8)
Stomach, Forestomach	15		15		15		15	
Epithelium, Hyperkeratosis	0		5*	(1.8)	1	(2.0)	1	(2.0)
Epithelium, Hyperplasia	2	(2.0)	5	(2.0)	2	(2.0)	2	(2.5)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

41-Week Drinking Water Study in Tg.AC Hemizygous Mice

Survival

Estimates of 41-week survival probabilities for male and female mice are shown in Table 17. Survival of exposed males and females was similar to that of the control groups.

Table 17
Survival of Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Animals initially in study	10	10	10	10
Moribund	0	1	0	0
Natural deaths	1	0	0	0
Animals surviving to study termination	9	9	10	10
Percent probability of survival at end of study[a]	90	90	100	100
Mean survival (days)[b]	262	268	281	281
Survival analysis[c]	P=0.459N	P=1.000N	P=1.000N	P=1.000N
Female				
Animals initially in study	10	10	10	10
Moribund	1	0	1	2
Natural deaths	2	1	2	0
Animals surviving to study termination	7	9	7	8
Percent probability of survival at end of study	70	90	70	80
Mean survival (days)	233	274	237	245
Survival analysis	P=1.000N	P=0.500N	P=1.000N	P=1.000N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 500, 1,000, and 2,000 mg/L males and females were generally less than those of the controls after weeks 35, 8, and 11 (males) and weeks 27, 28, and 26 (females), respectively (Figure 4; Tables 18 and 19). Water consumption by the 2,000 mg/L males and females was less than that by the control groups throughout the study (Tables H3 and H4). Drinking water concentrations of 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 76, 148, and 231 mg dichloroacetic acid/kg body weight to males and 92, 186, and 265 mg/kg to females. There were no chemical-related clinical findings.

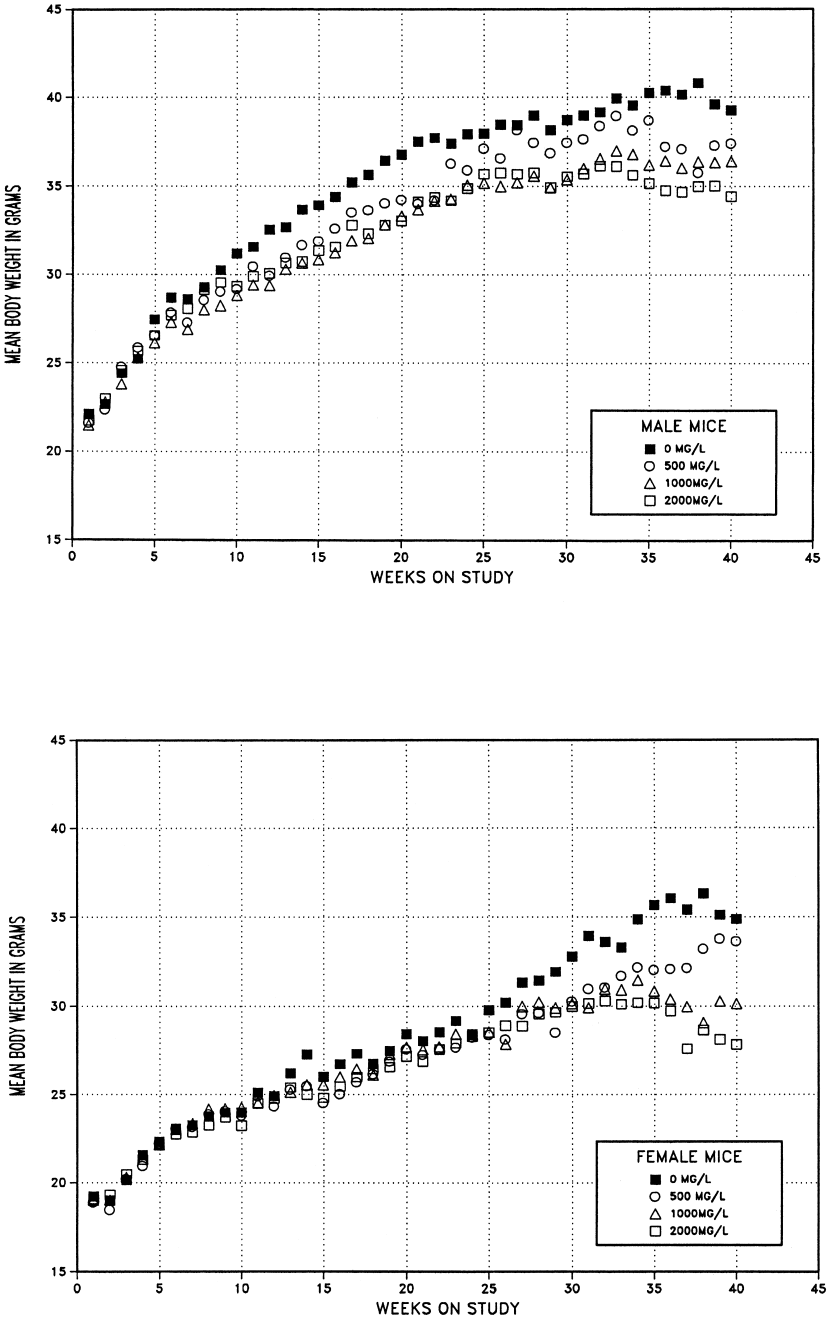


FIGURE 4
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Dichloroacetic Acid in Drinking Water for 41 Weeks

TABLE 18
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.1	10	21.6	98	10	21.5	97	10	21.7	98	10
2	22.7	10	22.3	98	10	22.8	100	10	23.0	101	10
3	24.4	10	24.8	102	10	23.8	98	10	24.6	101	10
4	25.2	10	25.9	103	10	25.3	100	10	25.7	102	10
5	27.5	10	26.6	97	10	26.2	95	10	26.6	97	10
6	28.7	10	27.8	97	10	27.3	95	10	27.7	97	10
7	28.6	10	27.3	96	10	26.9	94	10	28.1	98	10
8	29.3	10	28.6	98	10	28.0	96	10	29.1	99	10
9	30.3	10	29.0	96	10	28.2	93	10	29.6	98	10
10	31.2	10	29.2	94	10	28.8	92	10	29.3	94	10
11	31.6	10	30.5	97	10	29.4	93	10	29.9	95	10
12	32.5	10	29.9	92	10	29.4	91	10	30.1	93	10
13	32.7	10	31.0	95	10	30.3	93	10	30.7	94	10
14	33.7	9	31.7	94	10	30.7	91	10	30.8	91	10
15	33.9	9	31.9	94	10	30.9	91	10	31.4	93	10
16	34.4	9	32.6	95	10	31.2	91	10	31.6	92	10
17	35.2	9	33.5	95	10	31.9	91	10	32.8	93	10
18	35.6	9	33.7	95	10	32.1	90	10	32.3	91	10
19	36.4	9	34.1	94	10	32.8	90	10	32.8	90	10
20	36.8	9	34.2	93	10	33.3	91	10	33.1	90	10
21	37.5	9	34.0	91	10	33.7	90	10	34.1	91	10
22	37.7	9	34.2	91	10	34.2	91	10	34.4	91	10
23	37.4	9	36.3	97	9	34.3	92	10	34.2	91	10
24	37.9	9	35.9	95	9	35.1	93	10	34.9	92	10
25	38.0	9	37.1	98	9	35.2	93	10	35.7	94	10
26	38.5	9	36.6	95	9	35.0	91	10	35.8	93	10
27	38.4	9	38.2	100	9	35.2	92	10	35.7	93	10
28	39.0	9	37.4	96	9	35.6	91	10	35.8	92	10
29	38.2	9	36.9	97	9	34.9	91	10	34.9	91	10
30	38.7	9	37.4	97	9	35.4	92	10	35.6	92	10
31	39.0	9	37.6	96	9	36.0	92	10	35.7	92	10
32	39.2	9	38.4	98	9	36.6	93	10	36.1	92	10
33	39.9	9	39.0	98	9	37.0	93	10	36.1	91	10
34	39.5	9	38.1	97	9	36.8	93	10	35.6	90	10
35	40.2	9	38.7	96	9	36.2	90	10	35.2	88	10
36	40.4	9	37.2	92	9	36.4	90	10	34.8	86	10
37	40.1	9	37.1	93	9	36.0	90	10	34.7	87	10
38	40.8	9	35.8	88	9	36.4	89	10	35.0	86	10
39	39.6	9	37.3	94	9	36.4	92	10	35.0	88	10
40	39.2	9	37.4	95	9	36.4	93	10	34.4	88	10
Mean for weeks											
1-13	28.2		27.3	97		26.8	95		27.4	97	
14-40	38.0		36.0	95		34.7	91		34.4	91	

TABLE 19
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.2	10	18.9	98	10	19.0	99	10	19.1	100	10
2	19.0	10	18.5	97	10	19.0	100	10	19.3	102	10
3	20.2	10	20.2	100	10	20.3	101	10	20.5	102	10
4	21.6	10	21.0	97	10	21.3	99	10	21.3	99	10
5	22.3	10	22.2	100	10	22.1	99	10	22.2	100	10
6	23.1	10	23.1	100	10	23.0	100	10	22.7	98	10
7	23.3	10	23.1	99	10	23.3	100	10	22.8	98	10
8	23.8	10	23.9	100	10	24.2	102	10	23.2	98	10
9	24.0	10	24.1	100	10	24.2	101	10	23.7	99	10
10	24.0	10	23.8	99	10	24.3	101	10	23.2	97	10
11	25.1	10	24.9	99	10	24.5	98	10	24.5	98	10
12	24.9	10	24.3	98	10	25.0	100	9	24.8	100	10
13	26.2	10	25.3	97	10	25.1	96	9	25.4	97	10
14	27.3	10	25.5	93	10	25.6	94	9	25.0	92	9
15	26.0	9	24.5	94	10	25.5	98	9	24.8	95	9
16	26.7	9	25.0	94	10	26.0	97	9	25.5	96	9
17	27.3	9	25.7	94	10	26.4	97	9	26.0	95	8
18	26.7	8	26.2	98	10	26.1	98	9	26.5	99	8
19	27.5	8	26.9	98	10	27.3	99	9	26.6	97	8
20	28.4	8	27.6	97	10	27.7	98	9	27.2	96	8
21	28.1	8	27.2	97	10	27.5	98	8	26.9	96	8
22	28.6	7	27.6	97	10	27.7	97	8	27.6	97	8
23	29.2	7	27.7	95	10	28.4	97	8	27.9	96	8
24	28.4	7	28.2	99	10	28.4	100	8	28.3	100	8
25	29.8	7	28.4	95	10	28.6	96	8	28.5	96	8
26	30.2	7	28.1	93	10	27.9	92	8	28.9	96	8
27	31.3	7	29.6	95	10	30.0	96	7	28.9	92	8
28	31.5	7	29.6	94	10	30.2	96	7	29.6	94	8
29	31.9	7	28.5	89	10	29.9	94	7	29.7	93	8
30	32.8	7	30.3	92	9	30.1	92	7	30.0	92	8
31	34.0	7	31.0	91	9	29.9	88	7	30.2	89	8
32	33.6	7	31.0	92	9	31.0	92	7	30.3	90	8
33	33.3	7	31.7	95	9	30.9	93	7	30.1	90	8
34	34.9	7	32.2	92	9	31.5	90	7	30.2	87	8
35	35.7	7	32.0	90	9	30.8	86	7	30.2	85	8
36	36.1	7	32.1	89	9	30.4	84	7	29.7	82	8
37	35.4	7	32.1	91	9	30.0	85	7	27.6	78	8
38	36.3	7	33.2	92	9	29.1	80	7	28.7	79	8
39	35.1	7	33.8	96	9	30.3	86	7	28.1	80	8
40	34.9	7	33.6	96	9	30.1	86	7	27.9	80	8
Mean for weeks											
1-13	22.8		22.6	99		22.7	100		22.5	99	
14-40	31.1		29.2	94		28.8	93		28.2	91	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the forestomach, lung, liver, ovary, and thyroid gland. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables B5 through B8.

Forestomach: The incidence of multiple squamous cell papillomas in the 500 mg/L females was significantly increased compared to the controls (Tables 20 and B7). Papillomas were morphologically similar to that previously described in the 26-week dermal study.

Lung: The incidence of alveolar/bronchiolar adenoma in the 1,000 mg/L males was significantly increased compared to the controls (Tables 20 and B5). Two female mice exposed to 2,000 mg/L also had alveolar/bronchiolar adenomas of the lung (Tables 20 and B7)

Liver: The incidences of hepatocyte cytoplasmic vacuolization in all exposed groups were similar to those in the controls; however, there was an exposure-related increase in the mean severity (Tables 20, B6, and B8). Hepatocyte cytoplasmic vacuolization was morphologically similar to that previously described in the 26-week dermal study.

Ovary: The incidences of ovarian cysts were significantly increased in the 500 and 1,000 mg/L females compared to the controls (Tables 20 and B8).

Thyroid gland: The incidence of follicle degeneration in 500 mg/L males was significantly increased (Tables 20 and B6). Because the incidences in exposed females were not significantly increased (Table B8) and the incidences were not related to exposure concentration, the increase in the 500 mg/kg males was not considered related to exposure.

TABLE 20
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L		500 mg/L		1,000 mg/L		2,000 mg/L	
Male								
Lung[a,b]	10		10		10		10	
Alveolar/bronchiolar Adenoma (includes multiple)[c]	1		2		7**		3	
Liver	10		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic	9	(2.0)[d]	10	(2.3)	9	(3.2)	10	(3.8)
Thyroid Gland	10		10		10		10	
Follicle, Degeneration	3	(1.0)	9**	(1.1)	5	(1.2)	4	(1.0)
Female								
Lung	10		10		10		10	
Alveolar/bronchiolar Adenoma	0		0		0		2	
Stomach, Forestomach	10		10		10		10	
Squamous Cell Papilloma, Multiple	1		6*		4		4	
Squamous Cell Papilloma (includes multiple)	6		7		7		6	
Liver	10		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic	7	(2.0)	9	(2.6)	9	(2.9)	10	(3.0)
Ovary	10		10		10		10	
Cyst	0		7**	(2.0)	4*	(2.0)	1	(2.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Pulmonary neoplasms are not common in unexposed male Tg.AC hemizygous mice. In ten studies (including this study) for dermal, drinking water, and gavage routes of exposure, no pulmonary carcinomas were found at 39 to 43 weeks, and pulmonary adenomas were found in 4 of 112 (3.6%) Tg.AC hemizygous control mice.
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

26-Week Drinking Water Study in p53 Haploinsufficient Mice

Survival

Estimates of 26-week survival probabilities for male and female mice are shown in Table 21. Survival of all exposed groups was similar to that of the control groups.

Table 21
Survival of p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Animals initially in study	15	15	15	15
Animals surviving to study termination	15	15	15	15
Percent probability of survival at end of study[a]	100	100	100	100
Mean survival (days)[b]	177	177	177	177
Survival analysis[c]	—[d]	—	—	—
Female				
Animals initially in study	15	15	15	15
Moribund	0	0	0	1
Natural deaths	0	0	1	0
Animals surviving to study termination	15	15	14	14
Percent probability of survival at end of study	100	100	93	93
Mean survival (days)	178	178	177	177
Survival analysis	P=0.469	—	P=1.000	P=1.000

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns.
[d] Value of statistic cannot be computed.

Body Weights, Water and Compound Consumption, and Clinical Findings

The mean body weights of the 1,000 and 2,000 mg/L males after weeks 4 and 2, respectively, and those of 1,000 and 2,000 mg/L females after weeks 11 and 10, respectively, were less than those of the control groups (Tables 22 and 23; Figure 5). Water consumption by 1,000 and 2,000 mg/L males and females was less than that by the control groups throughout the study (Tables H5 and H6). Drinking water concentrations of 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 44, 83, and 149 mg/kg to males and 83, 144, and 222 mg/kg to females. No clinical findings were reported for male or female p53 haploinsufficient mice in the 26-week study.

Hematology

Hematology data are shown in Table E3. While minimal changes, some of which reached statistical significance, were seen, none were considered to be biologically relevant or related to dichloroacetic acid exposure.

TABLE 22
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.1	15	23.1	100	15	22.7	98	15	22.9	99	15
2	24.1	15	24.0	100	15	23.7	98	15	22.8	95	15
3	25.1	15	24.8	99	15	23.8	95	15	23.6	94	15
4	26.4	15	26.2	99	15	25.4	96	15	24.3	92	15
5	27.8	15	27.6	99	15	26.1	94	15	25.1	90	15
6	28.9	15	28.5	99	15	26.6	92	15	25.5	88	15
7	30.7	15	29.8	97	15	27.9	91	15	25.9	84	15
8	31.9	15	30.7	96	15	28.6	90	15	26.5	83	15
9	33.3	15	31.7	95	15	29.2	88	15	27.1	81	15
10	34.7	15	33.2	96	15	29.8	86	15	27.4	79	15
11	36.2	15	34.0	94	15	30.5	84	15	27.9	77	15
12	37.4	15	35.2	94	15	31.1	83	15	28.4	76	15
13	38.6	15	36.2	94	15	31.1	81	15	28.6	74	15
14	39.5	15	37.1	94	15	31.5	80	15	29.2	74	15
15	40.9	15	38.4	94	15	32.5	80	15	29.4	72	15
16	41.4	15	38.6	93	15	32.4	78	15	29.5	71	15
17	42.1	15	39.8	95	15	33.1	79	15	29.4	70	15
18	43.4	15	40.6	94	15	33.8	78	15	30.5	70	15
19	44.6	15	41.7	94	15	34.6	78	15	31.1	70	15
20	44.9	15	42.2	94	15	35.0	78	15	31.5	70	15
21	45.5	15	43.2	95	15	35.6	78	15	31.8	70	15
22	45.8	15	43.5	95	15	35.8	78	15	32.2	70	15
23	46.2	15	44.0	95	15	36.1	78	15	32.4	70	15
24	46.7	15	44.4	95	15	36.8	79	15	33.1	71	15
25	46.9	15	44.6	95	15	36.6	78	15	32.3	69	15
Mean for weeks											
1-13	30.6		29.6	97		27.4	90		25.8	84	
14-25	44.0		41.5	94		34.5	78		31.0	70	

TABLE 23
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.7	15	18.6	100	15	18.8	101	15	18.4	98	15
2	19.5	15	19.6	101	15	19.5	100	15	18.5	95	15
3	19.9	15	20.1	101	15	19.7	99	15	18.7	94	15
4	20.9	15	20.6	99	15	20.3	97	15	19.6	94	15
5	21.5	15	21.3	99	15	20.9	97	15	20.3	94	15
6	21.7	15	21.4	99	15	20.9	96	15	20.7	95	15
7	21.9	15	21.7	99	15	21.5	98	15	21.3	97	15
8	23.0	15	22.6	98	15	22.3	97	15	22.0	96	15
9	23.3	15	22.9	98	15	21.3	91	15	21.8	94	15
10	23.2	15	22.3	96	15	22.2	96	15	22.4	97	15
11	23.7	15	23.1	98	15	22.5	95	15	22.2	94	15
12	24.5	15	23.9	98	15	22.9	94	15	22.8	93	15
13	24.3	15	23.9	98	15	22.8	94	15	22.7	93	15
14	24.7	15	24.5	99	15	23.3	94	15	22.8	92	15
15	25.3	15	24.7	98	15	23.6	93	15	23.4	93	15
16	25.5	15	24.6	97	15	23.7	93	15	23.4	92	15
17	24.6	15	24.8	101	15	23.2	94	15	22.6	92	15
18	26.8	15	25.3	94	15	24.1	90	15	23.7	88	15
19	27.0	15	26.3	97	15	24.3	90	15	23.8	88	15
20	27.4	15	26.4	96	15	24.3	89	15	24.3	89	15
21	28.1	15	27.5	98	15	24.6	88	15	24.5	87	15
22	28.1	15	27.3	97	15	24.6	88	15	24.4	87	15
23	29.1	15	27.6	95	15	24.5	84	15	24.4	84	15
24	29.2	15	27.9	96	15	24.6	84	15	24.7	85	15
25	30.0	15	28.5	95	15	24.9	83	14	24.7	82	14
Mean for weeks											
1-13	22.0		21.7	99		21.2	96		20.9	95	
14-25	27.2		26.3	97		24.1	89		23.9	88	

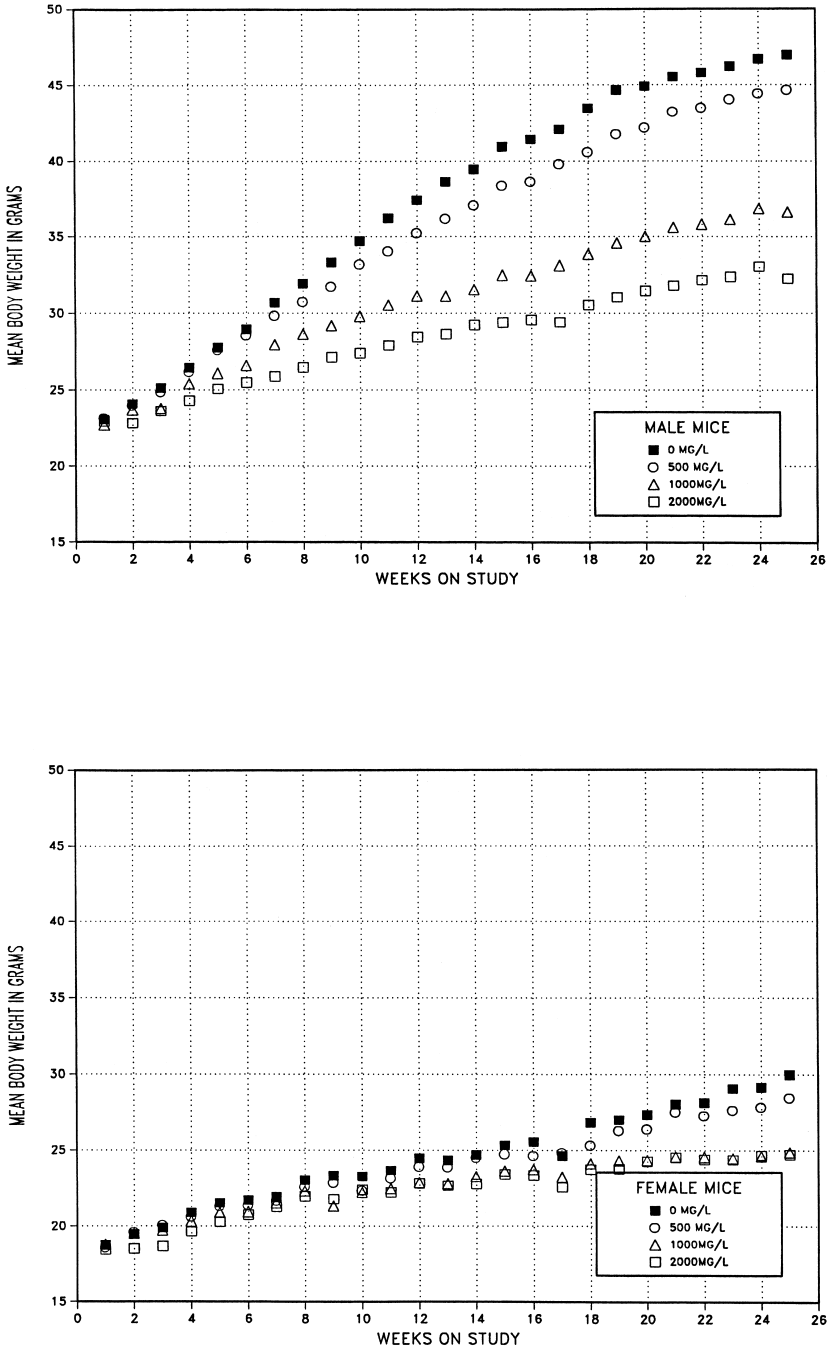


FIGURE 5
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Dichloroacetic Acid in Drinking Water for 26 Weeks

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver, pituitary gland, and thymus. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables C1 through C4.

Liver: The incidences of hepatocyte cytoplasmic vacuolization were significantly increased in all exposed groups of females (Tables 24 and C4). In both sexes, the average severity of cytoplasmic vacuolization generally increased with increasing exposure concentration. Although morphologically similar to lesions in the Tg.AC dermal and drinking water studies, the hepatocyte cytoplasmic vacuolization in the p53 haploinsufficient mice was generally more pronounced.

Pituitary gland: The incidence of pars distalis hyperplasia was significantly increased in the 1,000 mg/L males compared to the controls (Tables 24 and C2).

Thymus: The incidence of thymocyte necrosis was significantly increased in females exposed to 500 mg/L compared to the controls (Tables 24 and C4).

TABLE 24
Incidences of Selected Nonneoplastic Lesions in p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Liver[a]	15	15	15	15
Hepatocyte, Vacuolization Cytoplasmic[b]	15 (2.7)[c]	15 (3.4)	15 (3.4)	15 (4.0)
Pituitary Gland	15	15	15	15
Pars Distalis, Hyperplasia	0	0	6** (1.0)	0
Female				
Liver	15	15	15	15
Hepatocyte, Vacuolization Cytoplasmic	3 (1.0)	15** (2.2)	15** (3.1)	15** (3.5)
Thymus	15	15	15	15
Thymocyte, Necrosis	0	6** (1.8)	2 (2.0)	1 (2.0)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

41-Week Drinking Water Study in p53 Haploinsufficient Mice

Survival

Estimates of 41-week survival probabilities for male and female mice are shown in Table 25. Survival of all exposed groups was similar to that of the control groups.

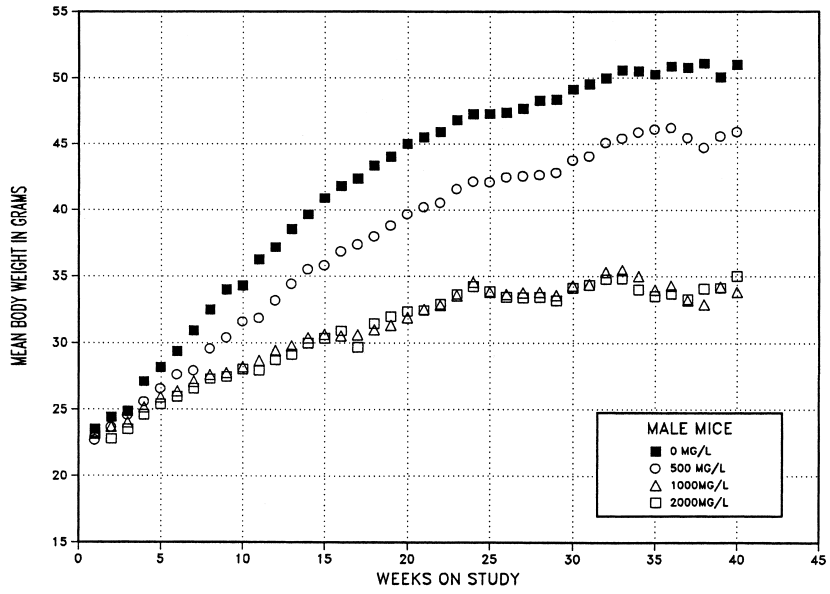
Table 25
Survival of p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
Animals initially in study	10	10	10	10
Natural deaths	1	0	1	0
Animals surviving to study termination	9	10	9	10
Percent probability of survival at end of study[a]	90	100	90	100
Mean survival (days)[b]	273	282	280	282
Survival analysis[c]	P=0.794N	P=1.000N	P=1.000N	P=1.000N
Female				
Animals initially in study	10	10	10	10
Moribund	0	1	0	0
Natural deaths	0	0	0	1
Animals surviving to study termination	10	9	10	9
Percent probability of survival at end of study	100	90	100	90
Mean survival (days)	283	282	283	275
Survival analysis	P=0.788	P=1.000	—[d]	P=1.000

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.
[d] Value of statistic cannot be computed.

Body Weights, Water and Compound Consumption, and Clinical Findings

The mean body weights of exposed groups of males were less than those of the controls after 4 (500 mg/L), 3 (1,000 mg/L), and 1 (2,000 mg/L) weeks on the study (Figure 6 and Table 26). The mean body weights of 1,000 and 2,000 mg/L females were less after week 9, and those of 500 mg/L females were less after week 27 (Figure 6 and Table 27). Water consumption by males and females exposed to 1,000 or 2,000 mg/L was less than that by the controls throughout the study (Tables H7 and H8). Drinking water concentrations of 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 45, 82, and 138 mg/kg to males and 65, 142, and 221 mg/kg to females. No chemical-related clinical findings were observed.



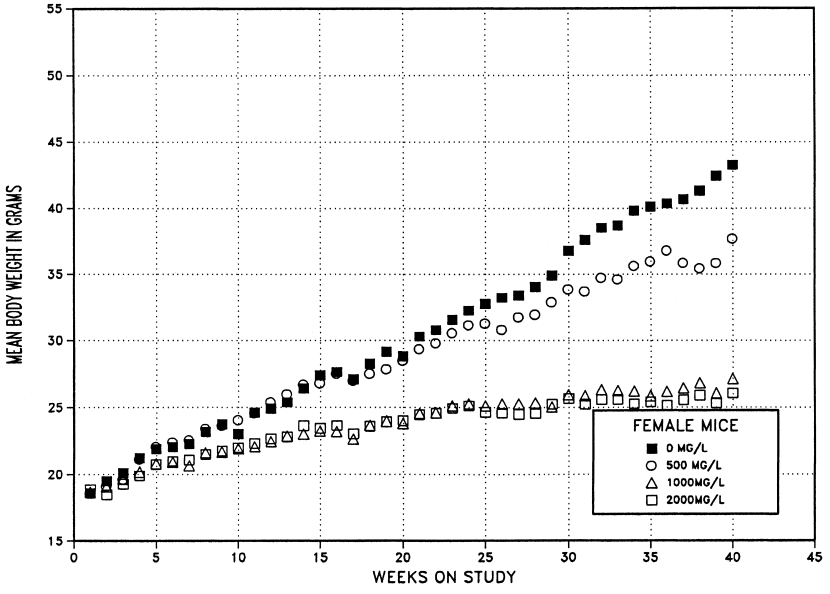


FIGURE 6
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Dichloroacetic Acid in Drinking Water for 41 Weeks

TABLE 26
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.5	10	22.7	97	10	23.3	99	10	23.2	99	10
2	24.4	10	23.7	97	10	23.7	97	10	22.8	93	10
3	24.9	10	24.6	99	10	24.0	96	10	23.5	94	10
4	27.1	10	25.6	95	10	25.2	93	10	24.6	91	10
5	28.2	10	26.6	94	10	25.9	92	10	25.4	90	10
6	29.4	10	27.6	94	10	26.4	90	10	26.0	88	10
7	30.9	10	27.9	90	10	27.1	88	10	26.6	86	10
8	32.5	10	29.6	91	10	27.6	85	10	27.3	84	10
9	34.0	10	30.4	89	10	27.8	82	10	27.5	81	10
10	34.3	10	31.6	92	10	28.2	82	10	28.0	82	10
11	36.3	10	31.9	88	10	28.7	79	10	28.0	77	10
12	37.2	10	33.2	89	10	29.4	79	10	28.8	77	10
13	38.6	10	34.4	89	10	29.8	77	10	29.2	76	10
14	39.7	10	35.5	89	10	30.4	77	10	30.0	76	10
15	40.9	10	35.8	88	10	30.6	75	10	30.4	74	10
16	41.8	10	36.9	88	10	30.5	73	10	30.9	74	10
17	42.4	10	37.4	88	10	30.6	72	10	29.7	70	10
18	43.4	10	38.0	88	10	31.0	71	10	31.5	73	10
19	44.0	10	38.8	88	10	31.3	71	10	32.0	73	10
20	45.0	10	39.7	88	10	31.9	71	10	32.4	72	10
21	45.5	10	40.2	88	10	32.5	71	10	32.4	71	10
22	45.9	10	40.5	88	10	32.8	72	10	32.9	72	10
23	46.8	10	41.6	89	10	33.6	72	10	33.7	72	10
24	47.3	10	42.2	89	10	34.5	73	10	34.2	72	10
25	47.3	10	42.1	89	10	33.8	72	10	33.8	72	10
26	47.4	10	42.5	90	10	33.6	71	10	33.4	71	10
27	47.7	10	42.6	89	10	33.8	71	10	33.4	70	10
28	48.3	9	42.7	88	10	33.8	70	10	33.4	69	10
29	48.4	9	42.8	88	10	33.6	69	10	33.2	69	10
30	49.1	9	43.8	89	10	34.3	70	10	34.1	70	10
31	49.5	9	44.1	89	10	34.4	70	10	34.4	70	10
32	50.0	9	45.1	90	10	35.3	71	10	34.8	70	10
33	50.6	9	45.4	90	10	35.5	70	10	34.8	69	10
34	50.5	9	45.9	91	10	35.0	69	10	34.0	67	10
35	50.3	9	46.1	92	10	34.0	68	10	33.5	67	10
36	50.9	9	46.2	91	10	34.3	67	10	33.7	66	10
37	50.8	9	45.5	90	10	33.2	65	10	33.3	66	10
38	51.1	9	44.7	88	10	32.9	64	10	34.1	67	10
39	50.0	9	45.6	91	10	34.2	68	9	34.1	68	10
40	51.0	9	45.9	90	10	33.8	66	9	35.0	69	10
Mean for weeks											
1-13	30.9		28.4	92		26.7	86		26.2	85	
14-40	47.2		42.1	89		33.2	70		33.1	70	

TABLE 27
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Weeks on Study	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.6	10	18.6	100	10	18.7	101	10	18.9	102	10
2	19.5	10	19.1	98	10	19.1	98	10	18.5	95	10
3	20.1	10	19.6	98	10	19.7	98	10	19.3	96	10
4	21.2	10	21.1	100	10	20.2	95	10	19.9	94	10
5	21.9	10	22.1	101	10	20.8	95	10	20.7	95	10
6	22.1	10	22.4	101	10	20.9	95	10	21.0	95	10
7	22.3	10	22.6	101	10	20.7	93	10	21.1	95	10
8	23.2	10	23.4	101	10	21.6	93	10	21.5	93	10
9	23.8	10	23.6	99	10	21.7	91	10	21.8	92	10
10	23.0	10	24.1	105	10	21.9	95	10	22.0	96	10
11	24.6	10	24.6	100	10	22.1	90	10	22.3	91	10
12	24.9	10	25.4	102	10	22.5	90	10	22.7	91	10
13	25.4	10	26.0	102	10	22.9	90	10	22.8	90	10
14	26.4	10	26.7	101	10	23.0	87	10	23.6	89	10
15	27.4	10	26.8	98	10	23.2	85	10	23.4	85	10
16	27.6	10	27.5	100	10	23.2	84	10	23.7	86	10
17	27.1	10	27.0	100	10	22.7	84	10	23.0	85	10
18	28.3	10	27.5	97	10	23.7	84	10	23.6	83	10
19	29.2	10	27.8	95	10	24.0	82	10	23.9	82	10
20	28.8	10	28.5	99	10	23.8	83	10	24.0	83	10
21	30.3	10	29.4	97	10	24.6	81	10	24.5	81	10
22	30.8	10	29.8	97	10	24.6	80	10	24.6	80	10
23	31.6	10	30.6	97	10	25.1	79	10	24.9	79	10
24	32.3	10	31.1	96	10	25.3	78	10	25.1	78	10
25	32.8	10	31.3	95	10	25.1	77	10	24.7	75	10
26	33.2	10	30.8	93	10	25.3	76	10	24.6	74	10
27	33.4	10	31.7	95	10	25.3	76	10	24.5	73	10
28	34.1	10	31.9	94	10	25.3	74	10	24.6	72	10
29	34.9	10	32.9	94	10	25.1	72	10	25.3	73	10
30	36.8	10	33.8	92	10	25.9	70	10	25.7	70	9
31	37.6	10	33.7	90	10	25.9	69	10	25.3	67	9
32	38.5	10	34.7	90	10	26.3	68	10	25.6	67	9
33	38.7	10	34.6	89	10	26.3	68	10	25.6	66	9
34	39.8	10	35.6	89	10	26.2	66	10	25.3	64	9
35	40.1	10	36.0	90	10	25.9	65	10	25.4	63	9
36	40.4	10	36.8	91	10	26.2	65	10	25.1	62	9
37	40.7	10	35.9	88	10	26.4	65	10	25.5	63	9
38	41.3	10	35.4	86	10	26.8	65	10	25.9	63	9
39	42.5	10	35.8	84	10	26.0	61	10	25.3	60	9
40	43.3	10	37.7	87	9	27.1	63	10	26.1	60	9
Mean for weeks											
1-13	22.4		22.5	100		21.0	94		21.0	94	
14-40	34.4		31.9	93		25.1	73		24.8	72	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver and ovary. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables C5 through C8.

Liver: The incidences of hepatocyte cytoplasmic vacuolization in exposed groups were similar to those in the controls; however, the average severity generally increased with increasing exposure concentration in females (Tables 28, C6, and C8). Hepatocellular adenoma was also observed in two 500 mg/L males (Tables 28 and C5). Hepatocyte cytoplasmic vacuolization was morphologically similar to that in the 26-week drinking water study in p53 haploinsufficient mice.

Ovary: The incidence of ovarian cyst in 2,000 mg/L females was significantly increased compared to the control group (Tables 28 and C8).

TABLE 28
Incidences of Selected Neoplasms and Nonneoplastic Lesions in p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L		500 mg/L		1,000 mg/L		2,000 mg/L	
Male								
Liver[a]	10		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic[b]	9	(3.6)[c]	10	(3.0)	10	(3.7)	10	(3.8)
Hepatocellular Adenoma	0		2		0		0	
Female								
Liver	10		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic	10	(1.9)	10	(2.7)	10	(3.7)	10	(3.6)
Ovary	10		10		10		10	
Cyst	0		1	(2.0)	1	(3.0)	7**	(2.0)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

GENETIC TOXICOLOGY

Dichloroacetic acid (concentration range of 33 to 6,666 μg/plate) was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 in the absence of S9 liver activation enzymes; no mutation induction was observed in either of these two base-pair substitution strains when exposure occurred in the presence of 30% rat or hamster liver S9 (Table D1). Dichloroacetic acid did not induce mutations in the *S. typhimurium* frame-shift strain TA98, with or without S9 (Table D1).

Dichloroacetic acid was tested for micronucleus induction in peripheral blood erythrocytes of male and female Tg.AC hemizygous and p53 haploinsufficient mice treated by dermal application (31.25 to 500 mg/kg) or in drinking water (500 to 2,000 mg/L) for 26 weeks (Tables D2 through D4). No induction of micronuclei was seen in Tg.AC hemizygous mice treated by either route or in the p53 haploinsufficient mice, which were exposed only by drinking water. The percentages of polychromatic erythrocytes were not significantly altered by chemical treatment.

Peripheral blood samples from B6C3F_1 mice exposed to dichloroacetic acid in drinking water (67 to 1,000 mg/L) were analyzed for frequency of micronucleated normochromatic erythrocytes at the end of a 3-month exposure period; no increases in micronucleated erythrocytes were seen in male mice, but a small increase was observed in females (Table D5). The increase in frequency of micronucleated erythrocytes in the female mice was judged to be equivocal due to a positive trend test (P=0.007), but none of the individual dosed groups differed significantly from the control group. The percentages of polychromatic erythrocytes were not significantly altered by chemical treatment.

DISCUSSION AND CONCLUSIONS

Dichloroacetic acid is a disinfection by-product of the chlorination of drinking water (Weisel *et al.*, 1999). Dichloroacetic acid is commonly found in the drinking water at concentrations between 5µg/L and 125 µg/L (IARC, 1995). Concentrations of dichloroacetic acid tend to decline with length of time in the distribution system and are higher in the spring and summer (IARC, 2004; Rodriguez *et al.*, 2004). In eight studies, dichloroacetic acid administered in the drinking water to male and/or female mice increased the incidences of hepatocellular adenomas and/or carcinomas (IARC, 2004). Dichloroacetic acid at very high doses increased the incidence of hepatocellular carcinomas in the Fisher 344 rat (DeAngelo *et al.*, 1996). In three studies in carcinogen-initiated male and female mice, dichloroacetic acid exposure in the drinking water promoted the formation of hepatocellular carcinomas (IARC, 2004). While dichloroacetic acid is considered carcinogenic to animals (IARC, 2004), carcinogenic activity is usually seen at 1 g/L doses or greater. As one of the most common haloacetic acids in the drinking water, dichloroacetic acid was nominated to the NTP by the United States Environmental Protection Agency (EPA) for toxicity and carcinogenicity studies in transgenic mice to provide additional information in support of EPA drinking water regulations (40 CFR Part 141). A primary goal was to determine whether transgenic mouse models could prove effective in either hazard identification or in prioritizing which disinfection by-products warranted further research (Fawell *et al.*, 1997; Boorman, 1999). Dichloroacetic acid was considered a good test chemical because of the amount of comparative data available in both standard and carcinogen-initiated mice.

The combination of Tg.AC hemizygous mice and p53 haploinsufficient mice has been suggested as an effective means of identifying chemical carcinogens and assessing potential risk (Tennant *et al.*, 1995). Tg.AC hemizygous mice were reported to respond to tumor promoters, mutagenic chemicals, and nonmutagenic chemicals while the p53 haploinsufficient mice responded to mutagens within 6 months allowing for the testing of more chemicals

within a shorter period of time (Cannon *et al.*, 1997). We evaluated dichloroacetic acid in drinking water because of the common occurrence of this chemical in drinking water (IARC, 2004). Dermal studies were also included for Tg.AC hemizygous mice since it was reported that tumors usually occurred within 10 weeks of initiation of exposure (Tennant *et al.*, 1995). A visually observable and quantitative tumor response could allow evaluation of either individual chemicals or drinking water by-product mixtures as they might be found with different disinfection processes in a very rapid and cost-effective manner.

In the 26-week study, 2 of 15 high dose (500 mg/kg) males and 2 of 15 high dose (500 mg/kg) females had squamous cell papillomas at the site of dermal application. No papillomas were found at the site of application in males or females in the lowest dose groups (31.25 mg/kg) at either 26 or 39 weeks. There was a significant increase in the incidence of papillomas at the site of application in 500 mg/kg males (8/10) and females (6/10) at 39 weeks compared to none in the vehicle controls for males and females. The increase in tumor multiplicity was modest with a mean of 2.6 tumors per tumor-bearing mouse in 500 mg/kg males and 1.4 tumors per tumor-bearing mouse in males at 39 weeks. For comparison, there were approximately 20 tumors per tumor-bearing male or female mouse by 26 weeks in the positive control groups receiving 12-*O*-tetradecanoylphorbol-13-acetate by dermal application.

The Tg.AC hemizygous mouse is sensitive to dermal injury (Cannon *et al.*, 1997). Following a single full thickness wound, all of the mice develop papillomas with approximately five papillomas per mouse by 10 weeks (Battalora *et al.*, 2001). The dichloroacetic acid exposure caused a dose-related hyperkeratosis and epithelial hyperplasia at the site of application consistent with mild injury. It is unlikely that the papillomas seen at the site of application were secondary to the irritating nature of dichloroacetic acid. The severity of the hyperkeratosis and hyperplasia was generally minimal to mild. In a previous NTP study, rotenone applied to the skin caused hyperplasia, hyperkeratosis, and inflammation at the site of application but failed to increase the incidence of dermal papillomas (Eastin *et al*., 1998). In a preliminary study using FVB/N mice we demonstrated that

dichloroacetic acid is readily absorbed through the skin with dichloroacetic plasma concentrations exceeding 20 µg/mL within 15 minutes after a single dermal application of 500 mg/kg in acetone (NIEHS, 1999). There was no increase in the incidence of dermal papillomas at sites other than the site of application.

Pulmonary adenomas were statistically increased in the male mice in the drinking water study (discussed below) prompting a review of the dermal study for the occurrence of pulmonary adenomas. While not reaching statistical significance, nine pulmonary adenomas (9/15, 6%) occurred in the male and female mice exposed to dichloroacetic acid dermally versus one (1/15, 2%) in the control groups. Eight of the pulmonary adenomas occurred in the two highest dermal exposure groups providing additional evidence that these tumors may be chemical-related. While there were never more than two mice with pulmonary adenomas in any dose group, the small group sizes, the uncommon occurrence of these tumors in untreated mice, and the significance of the occurrence of pulmonary adenomas in the companion drinking water study suggest that dermal dichloroacetic acid exposure may cause or promote pulmonary adenomas in Tg.AC hemizygous mice.

It has been established in eight studies that dichloroacetic acid exposure in drinking water causes hepatocellular tumors in more traditional mouse models (IARC, 2004). In the current Tg.AC hemizygous mouse dermal study, the response was quite weak with the first dermal papillomas appearing at 25 to 26 weeks and with a very modest response in tumor multiplicity. This suggests that the Tg.AC hemizygous mouse model is not robust enough to determine which haloacetic acids may be tumorigenic in mice.

Twenty six- and 41-week drinking water studies were conducted in Tg.AC hemizygous mice at dichloroacetic acid exposure concentrations of 500 1,000, or 2,000 mg/L. These drinking water concentrations have been shown to increase liver tumors in male and female $B6C3F_1$ mice (Bull *et al.*, 1990; DeAngelo *et al.*, 1999). The hepatocellular adenoma found in one 2,000 mg/L male at 41 weeks was considered unrelated to treatment. There were no hepatocellular adenomas found in the females nor preneoplastic lesions of the liver in either males or females at 26 or 41 weeks. There was an increased incidence of pulmonary adenoma in males exposed to

1,000 mg/L for 41 weeks (7/10) compared to the incidence of pulmonary adenomas (1/10) in the control group. However, the incidence of pulmonary adenoma did not increase (3/10) at the higher exposure of 2,000 mg/L in the 41-week study. Survival was similar between the 1,000 and 2,000 mg/L groups, but mean body weight was slightly lower in the 2,000 mg/L males (mean body weight of 34.4 g) than in the 1,000 mg/L males (mean body weight of 36.4 g). The pulmonary carcinoma in one 1,000 mg/L male at 26 weeks appears to add some support to the finding as does the occurrence of two pulmonary adenomas in the 2,000 mg/L females in the 41-week study. While the incidences of neoplastic and nonneoplastic lesions were not monotonically dose related, the occurrence of seven uncommon pulmonary adenomas in the 1,000 mg/L dose group and 3 in the top dose group were considered related to dichloroacetic acid exposure. The clear positive liver tumor response in several mouse dichloroacetic acid drinking water studies (IARC, 2004) suggests that the Tg.AC mouse is less sensitive than traditional mouse models using the drinking water route of exposure. The pulmonary adenoma response was significant only in the mid-dose group in male mice, and the Tg.AC hemizygous mouse model completely fails to predict the mouse liver tumor response seen with traditional animal models.

Twenty six- and 41-week drinking water studies were also conducted at the same concentrations (500 1,000, and 2,000 mg/L) in p53 haploinsufficient mice. There were no increased neoplasm incidences in either sex for either study. Two hepatocellular adenomas were found in the 500 mg/L males at 41 weeks and were considered unrelated to treatment, because liver neoplasms were not found at the higher exposures in males. Further, preneoplastic hepatic lesions were not found in any mice at any exposure concentration. In this study, dichloroacetic acid appeared only weakly mutagenic in *Salmonella typhimurium* consistent with the findings of others (Harrington-Brock *et al.*, 1998; Kargalioglu *et al.*, 2002). Dichloroacetic acid exposure also did not induce micronuclei in peripheral blood of either Tg.AC hemizygous or in p53 haploinsufficient mice. Because the p53 haploinsufficient mice are considered unresponsive to nonmutagens, the negative results with this weakly mutagenic chemical may not be surprising. Furthermore, the p53 haploinsufficient mouse is also often unresponsive to hepatocarcinogens (Finnberg *et al.*, 2004; Iidaka *et al.*, 2005) but does show increased rates of liver neoplasms with dietary administration of kojic acid (a fungal toxin) (Takizawa *et al.*, 2003). In a companion drinking water study, the p53 haploinsufficient mouse failed to respond to high levels of bromodichloromethane, a

multisite, multispecies carcinogen, in the drinking water or by gavage (NTP, 2005). The data from the current study and from the bromodichloromethane study (NTP, 2005) suggest that the p53 haploinsufficient mouse is not a suitable model for detecting possible hazardous disinfection by-products in drinking water. Thus our attempt to find a genetically modified mouse model that could serve as a rapid and reliable screen for assessing the potential toxicity of the different haloacetic acids that occur in the drinking water was not realized. The Tg.AC hemizygous mouse proved to be insensitive to dichloroacetic acid by either dermal or drinking water exposure while the p53 haploinsufficient mouse model was unresponsive to dichloroacetic acid by drinking water exposure. Since haloacetic acids generally occur in the low µg/L range in the drinking water, it is unlikely that either mouse model evaluated will have much utility in assessing the relative toxicity of the different members of the family of haloacetic acids as they are found in U.S. water supplies.

CONCLUSIONS

Under the conditions of these drinking water studies, there was *no evidence of carcinogenic activity** of dichloroacetic acid in male or female p53 haploinsufficient mice exposed to 0, 500, 1,000, or 2,000 mg/L for 26 or 41 weeks. The incidences and/or severities of cytoplasmic vacuolization of the hepatocyte were increased in males and females exposed to dichloroacetic acid for 26 or 41 weeks.

Under the conditions of these dermal studies, there were increased incidences of squamous cell papillomas at the site of application in male and female Tg.AC hemizygous mice exposed to 500 mg/kg for 39 weeks. There were dose-related increased incidences of epidermal hyperkeratosis and hyperplasia at the site of application in both male and female mice exposed to dichloroacetic acid for 26 or 39 weeks.

Under the conditions of these drinking water studies, there was an increase in the incidence of alveolar/bronchiolar adenoma in male Tg.AC hemizygous mice exposed to 1,000 mg/L for 41 weeks. There were a few bronchiolar/alveolar carcinomas in males and females exposed to dichloroacetic acid in the drinking water for 26 weeks and a few bronchiolar/alveolar adenomas in females exposed to dichloroacetic acid in the drinking water for 41 weeks.

There were increased incidences and/or severities of cytoplasmic vacuolization of the hepatocyte in male and female Tg.AC hemizygous mice exposed to dichloroacetic acid in the drinking water study for 26 or 41 weeks.

The marginally increased incidences of pulmonary adenomas and/or carcinomas compared to the unexposed groups found in both the dermal and drinking water studies at 26, 39, or 41 weeks were considered to be related to dichloroacetic acid exposure.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13.

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APPENDIX A
SUMMARY OF LESIONS
IN Tg.AC HEMIZYGOUS MICE
IN THE DERMAL STUDIES
OF DICHLOROACETIC ACID

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2			2
Natural deaths		1	1	1
Survivors				
Terminal sacrifice	13	14	14	12
Animals examined microscopically	15	15	15	15
Alimentary System				
Salivary glands	(1)			
Carcinoma	1 (100%)			
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma		4 (27%)	3 (20%)	3 (20%)
Squamous cell papilloma, multiple	1 (7%)	2 (13%)		
Tooth	(2)	(3)	(1)	(4)
Odontogenic tumor	2 (100%)	3 (100%)	1 (100%)	4 (100%)
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Squamous cell papilloma	5 (33%)	3 (20%)	2 (13%)	3 (20%)
Squamous cell papilloma, multiple		2 (13%)	1 (7%)	2 (13%)
Site of application, keratoacanthoma				1 (7%)
Site of application, squamous cell papilloma			1 (7%)	1 (7%)
Site of application, squamous cell papilloma, multiple				1 (7%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma	1 (7%)		1 (7%)	

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[b]	7	11	8	8
Total primary neoplasms	10	14	9	15
Total animals with benign neoplasms	6	10	8	7
Total benign neoplasms	7	11	8	11
Total animals with malignant neoplasms	1			
Total malignant neoplasms	1			
Total animals with uncertain neoplasms-benign or malignant	2	3	1	4
Total uncertain neoplasms	2	3	1	4

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2			2
Natural deaths		1	1	1
Survivors				
Terminal sacrifice	13	14	14	12
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	3 (20%)			
Inflammation, chronic active	2 (13%)	8 (53%)	4 (27%)	2 (13%)
Pigmentation				1 (7%)
Hepatocyte, necrosis	1 (7%)	2 (13%)		2 (13%)
Hepatocyte, vacuolization cytoplasmic	3 (20%)	4 (27%)	14 (93%)	15 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Epithelium, hyperkeratosis				1 (7%)
Epithelium, hyperplasia	1 (7%)			1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hypertrophy	11 (73%)	7 (47%)	7 (47%)	7 (47%)
Subcapsular, hyperplasia	4 (27%)	1 (7%)	2 (13%)	
Parathyroid gland		(1)		
Cyst		1 (100%)		
Pituitary gland	(15)	(15)	(14)	(15)
Pars distalis, cyst		1 (7%)		3 (20%)
Thyroid gland	(15)	(15)	(15)	(15)
Follicle, degeneration	3 (20%)	3 (20%)	2 (13%)	1 (7%)
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Degeneration	2 (13%)	1 (7%)	1 (7%)	1 (7%)
Inflammation, chronic active				1 (7%)
Testes	(15)	(15)	(15)	(15)
Cyst		1 (7%)	1 (7%)	1 (7%)
Germinal epithelium, degeneration	2 (13%)	1 (7%)	1 (7%)	2 (13%)
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(14)
Hyperplasia, lymphoid		2 (13%)		1 (7%)
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	3 (20%)			
Thymus	(15)	(15)	(15)	(15)
Atrophy	1 (7%)	1 (7%)		2 (13%)
Cyst	6 (40%)	1 (7%)	4 (27%)	2 (13%)
Thymocyte, necrosis				2 (13%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Epidermis, hyperplasia	2 (13%)	3 (20%)	2 (13%)	3 (20%)
Site of application, epidermis, hyperkeratosis	2 (13%)	7 (47%)	15 (100%)	14 (93%)
Site of application, epidermis, hyperplasia		2 (13%)	11 (73%)	13 (87%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation, chronic active		1 (7%)		1 (7%)
Pigmentation				1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Nephropathy	7 (47%)	7 (47%)	11 (73%)	13 (87%)
Renal tubule, dilatation	4 (27%)	2 (13%)	2 (13%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund		1		
Natural deaths	4	2	1	
Survivors				
Terminal sacrifice	11	12	14	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands	(1)			
Carcinoma	1 (100%)			
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	4 (27%)	5 (33%)	4 (27%)	5 (33%)
Squamous cell papilloma, multiple	3 (20%)	1 (7%)	4 (27%)	2 (13%)
Tooth	(5)	(2)	(5)	(2)
Odontogenic tumor	5 (100%)	2 (100%)	5 (100%)	2 (100%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Adrenal medulla	(15)	(15)	(15)	(15)
Pituitary gland	(15)	(15)	(15)	(15)
Hematopoietic System				
Spleen	(15)	(15)	(15)	(15)
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Squamous cell papilloma	6 (40%)	5 (33%)	6 (40%)	2 (13%)
Squamous cell papilloma, multiple		3 (20%)	2 (13%)	
Site of application, squamous cell papilloma				2 (13%)
Respiratory System				
Lung	(15)	(15)	(15)	(14)
Alveolar/bronchiolar adenoma		1 (7%)	1 (7%)	
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)			1 (7%)

TABLE A3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	12	12	10
Total primary neoplasms	20	17	22	14
Total animals with benign neoplasms	8	11	11	10
Total benign neoplasms	13	15	17	11
Total animals with malignant neoplasms	2			1
Total malignant neoplasms	2			1
Total animals with uncertain neoplasms-benign or malignant	5	2	5	2
Total uncertain neoplasms	5	2	5	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund		1		
Natural deaths	4	2	1	
Survivors				
Terminal sacrifice	11	12	14	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Inflammation, chronic active	11 (73%)	13 (87%)	14 (93%)	11 (73%)
Hepatocyte, necrosis	1 (7%)			3 (20%)
Hepatocyte, vacuolization cytoplasmic	6 (40%)	4 (27%)	14 (93%)	15 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Epithelium, hyperkeratosis	1 (7%)			
Epithelium, hyperplasia	1 (7%)	1 (7%)	1 (7%)	
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Accessory adrenal cortical nodule		2 (13%)	7 (47%)	4 (27%)
Subcapsular, hyperplasia	5 (33%)	8 (53%)	6 (40%)	9 (60%)
Pituitary gland	(15)	(15)	(15)	(15)
Pars distalis, cyst	3 (20%)	4 (27%)	3 (20%)	3 (20%)
Thyroid gland	(15)	(15)	(15)	(15)
Ectopic thymus		1 (7%)		
Follicle, degeneration	5 (33%)	4 (27%)	2 (13%)	1 (7%)
Follicular cell, hyperplasia			1 (7%)	
Genital System				
Ovary	(15)	(15)	(15)	(15)
Cyst	3 (20%)	2 (13%)	1 (7%)	1 (7%)
Uterus	(15)	(15)	(15)	(15)
Endometrium, hyperplasia, cystic	10 (67%)	12 (80%)	10 (67%)	14 (93%)
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(15)
Hyperplasia, lymphoid	3 (20%)	1 (7%)	4 (27%)	2 (13%)
Thymus	(15)	(15)	(15)	(15)
Atrophy	3 (20%)	1 (7%)	1 (7%)	
Cyst	3 (20%)	8 (53%)	7 (47%)	9 (60%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Hyperkeratosis	1 (7%)		1 (7%)	
Inflammation, chronic active	1 (7%)			
Epidermis, hyperplasia	3 (20%)	1 (7%)	1 (7%)	2 (13%)
Site of application, epidermis, hyperkeratosis	8 (53%)	9 (60%)	14 (93%)	14 (93%)
Site of application, epidermis, hyperplasia		1 (7%)	10 (67%)	13 (87%)
Respiratory System				
Lung	(15)	(15)	(15)	(14)
Inflammation, chronic active	1 (7%)			1 (7%)
Thrombosis				1 (7%)
Alveolar epithelium, hyperplasia			1 (7%)	1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Nephropathy	3 (20%)	6 (40%)	5 (33%)	5 (33%)
Renal tubule, dilatation	2 (13%)	3 (20%)	3 (20%)	1 (7%)
Renal tubule, hyperplasia			1 (7%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1	3	1	2
Natural deaths		1	1	1
Survivors				
Terminal sacrifice	9	6	8	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Pancreas	(1)			
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	2 (20%)		4 (40%)
Squamous cell papilloma, multiple	5 (50%)	4 (40%)	8 (80%)	3 (30%)
Tooth	(1)	(4)	(2)	(2)
Odontogenic tumor	1 (100%)	4 (100%)	2 (100%)	1 (50%)
Odontogenic tumor, multiple				1 (50%)
Hematopoietic System				
Lymph node	(1)	(1)		
Lymph node, mandibular	(10)	(10)	(10)	(10)
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)
Thymus	(9)	(10)	(10)	(9)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)			7 (70%)
Squamous cell papilloma, multiple	6 (60%)	4 (40%)	7 (70%)	2 (20%)
Site of application, squamous cell papilloma			1 (10%)	2 (20%)
Site of application, squamous cell papilloma, multiple			1 (10%)	6 (60%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma			1 (10%)	2 (20%)
Nose				(1)
Glands, carcinoma				1 (100%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)

TABLE A5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic	1 (10%)			
Lymphoma malignant		1 (10%)		
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	10	9	10
Total primary neoplasms	17	15	20	29
Total animals with benign neoplasms	10	7	9	10
Total benign neoplasms	15	10	18	26
Total animals with malignant neoplasms	1	1		1
Total malignant neoplasms	1	1		1
Total animals with uncertain neoplasms-benign or malignant	1	4	2	2
Total uncertain neoplasms	1	4	2	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1	3	1	2
Natural deaths		1	1	1
Survivors				
Terminal sacrifice	9	6	8	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Cyst			1 (10%)	
Inflammation, chronic active	6 (60%)	7 (70%)	5 (50%)	5 (50%)
Mineralization	1 (10%)			
Hepatocyte, necrosis	1 (10%)		2 (20%)	6 (60%)
Hepatocyte, vacuolization cytoplasmic	9 (90%)	7 (70%)	8 (80%)	10 (100%)
Mesentery	(1)		(2)	
Fat, fibrosis			2 (100%)	
Fat, mineralization	1 (100%)		1 (50%)	
Fat, necrosis	1 (100%)		2 (100%)	
Salivary glands			(1)	(1)
Atrophy			1 (100%)	1 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Epithelium, hyperkeratosis		1 (10%)		
Epithelium, hyperplasia		1 (10%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule				1 (10%)
Hypertrophy	8 (80%)	8 (80%)	6 (60)	7 (70%)
Subcapsular, hyperplasia		1 (10%)	1 (10%)	1 (10%)
Pituitary gland	(9)	(10)	(9)	(10)
Pars distalis, cyst		2 (20%)	1 (11%)	
Thyroid gland	(10)	(10)	(10)	(10)
Follicle, degeneration	2 (20%)	2 (20%)	2 (20%)	2 (20%)
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Degeneration	3 (30%)	2 (20%)	1 (10%)	1 (10%)
Preputial gland	(1)			
Duct, ectasia	1 (100%)			
Testes	(10)	(10)	(10)	(10)
Cyst	3 (30%)	1 (10%)	1 (10%)	
Germinal epithelium, degeneration	3 (30%)	2 (20%)	1 (10%)	8 (80%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid		3 (30%)	1 (10%)	1 (10%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)		
Thymus	(9)	(10)	(10)	(9)
Atrophy		3 (30%)	1 (10%)	4 (44%)
Cyst	3 (33%)	4 (40%)	2 (20%)	3 (33%)
Thymocyte, necrosis			1 (10%)	1 (11%)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Cyst epithelial inclusion			1 (10%)	
Hyperplasia			1 (10%)	1 (10%)
Inflammation, chronic active				2 (20%)
Epidermis, hyperplasia, focal	3 (30%)	1 (10%)		
Sebaceous gland, cyst		1 (10%)		
Site of application, dermis, inflammation, chronic active				1 (10%)
Site of application, epidermis, hyperkeratosis	2 (20%)	8 (80%)	9 (90%)	10 (100%)
Site of application, epidermis, hyperplasia			8 (80%)	9 (90%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar epithelium, hyperplasia				1 (10%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst	2 (20%)		1 (10%)	
Infiltration cellular, plasma cell		1 (10%)		
Mineralization	2 (20%)			
Nephropathy	2 (20%)	7 (70%)	7 (70%)	8 (80%)
Glomerulus, inflammation, membranoproliferative	1 (10%)			
Renal tubule, dilatation	1 (10%)	4 (40%)	4 (40%)	4 (40%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	2	3	2	2
Natural deaths		2	2	
Survivors				
Terminal sacrifice	8	5	6	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Salivary glands		(1)	(1)	
Carcinoma		1 (100%)	1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	3 (30%)		1 (10%)
Squamous cell papilloma, multiple	4 (40%)	5 (50%)	5 (50%)	6 (60%)
Tooth	(4)	(2)	(3)	(3)
Odontogenic tumor	4 (100%)	2 (100%)	3 (100%)	1 (33%)
Odontogenic tumor, multiple				1 (33%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)	(10)	(9)	(10)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Uterus	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Hematopoietic System				
Lymph node, mandibular	(10)	(9)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Spleen	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Squamous cell carcinoma	1 (10%)			
Squamous cell papilloma	2 (20%)	3 (30%)	1 (10%)	3 (30%)
Squamous cell papilloma, multiple	5 (50%)	2 (20%)	4 (40%)	4 (40%)
Site of application, squamous cell papilloma				3 (30%)
Site of application, squamous cell papilloma, multiple				3 (30%)

TABLE A7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma			1 (10%)	2 (20%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Lymphoma malignant	1 (10%)			
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	8	9	9
Total primary neoplasms	19	16	16	24
Total animals with benign neoplasms	8	8	6	9
Total benign neoplasms	13	13	11	22
Total animals with malignant neoplasms	2	1	2	
Total malignant neoplasms	2	1	2	
Total animals with uncertain neoplasms-benign or malignant	4	2	3	2
Total uncertain neoplasms	4	2	3	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	2	3	2	2
Natural deaths		2	2	
Survivors				
Terminal sacrifice	8	5	6	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Inflammation, chronic active	9 (90%)	9 (90%)	8 (80%)	10 (100%)
Hepatocyte, necrosis	2 (20%)	1 (10%)		5 (50%)
Hepatocyte, vacuolization cytoplasmic	7 (70%)	6 (60%)	8 (80%)	10 (100%)
Salivary glands		(1)	(1)	
Degeneration		1 (100%)		
Tooth	(4)	(2)	(3)	(3)
Inflammation, chronic				1 (33%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	3 (30%)		2 (20%)	
Mineralization	3 (30%)	1 (10%)	2 (20%)	
Subcapsular, hyperplasia	7 (70%)	6 (60%)	8 (80%)	5 (50%)
Pituitary gland	(10)	(10)	(9)	(10)
Pars distalis, cyst	1 (10%)	2 (20%)	2 (22%)	
Thyroid gland	(10)	(10)	(10)	(10)
Follicle, degeneration	2 (20%)	6 (60%)	2 (20%)	
Genital System				
Ovary	(10)	(10)	(10)	(10)
Cyst	2 (20%)	1 (10%)	1 (10%)	2 (20%)
Uterus	(10)	(10)	(10)	(10)
Inflammation, chronic active	1 (10%)			
Endometrium, hyperplasia, cystic	9 (90%)	8 (80%)	8 (80%)	8 (80%)
Hematopoietic System				
Lymph node, mandibular	(10)	(9)	(10)	(10)
Hyperplasia, lymphoid	2 (20%)	3 (33%)	3 (30%)	2 (20%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			1 (10%)	
Thymus	(10)	(10)	(9)	(10)
Atrophy	3 (30%)	3 (30%)	2 (22%)	1 (10%)
Cyst	5 (50%)	2 (20%)	2 (22%)	3 (30%)
Thymocyte, necrosis		1 (10%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Fibrosis			1 (10%)	
Hyperkeratosis			1 (10%)	
Inflammation, chronic active			3 (30%)	
Ulcer			2 (20%)	
Epidermis, hyperplasia, focal		1 (10%)	2 (20%)	
Site of application, epidermis, hyperkeratosis	5 (50%)	8 (80%)	9 (90%)	10 (100%)
Site of application, epidermis, hyperplasia			3 (30%)	6 (60%)
Site of application, epidermis, inflammation, chronic active			1 (10%)	
Nervous System				
Spinal cord				(1)
Necrosis				1 (100%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation, chronic active	2 (20%)			
Alveolar epithelium, hyperplasia				1 (10%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy	4 (40%)	2 (20%)	3 (30%)	2 (20%)
Glomerulus, inflammation, membranoproliferative	1 (10%)			
Renal tubule, dilatation	1 (10%)	2 (20%)	1 (10%)	1 (10%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Special Senses System

APPENDIX B
SUMMARY OF LESIONS
IN Tg.AC HEMIZYGOUS MICE
IN THE DRINKING WATER STUDIES
OF DICHLOROACETIC ACID

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1	2	4	1
Survivors				
Terminal sacrifice	14	13	11	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands			(1)	(1)
Carcinoma			1 (100%)	1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	1 (7%)	3 (20%)	5 (33%)	3 (20%)
Squamous cell papilloma, multiple	3 (20%)	2 (13%)	2 (13%)	3 (20%)
Tooth	(1)	(3)	(4)	
Odontogenic tumor	1 (100%)	3 (100%)	3 (75%)	
Odontogenic tumor, multiple			1 (25%)	
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Adrenal medulla	(15)	(15)	(15)	(15)
Pituitary gland	(14)	(15)	(15)	(15)
Hematopoietic System				
Spleen	(15)	(15)	(15)	(15)
Integumentary System				
Skin	(4)	(4)	(4)	(6)
Squamous cell papilloma	2 (50%)	3 (75%)	2 (50%)	3 (50%)
Squamous cell papilloma, multiple		1 (25%)	2 (50%)	1 (17%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar carcinoma			1 (7%)	
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic		1 (7%)		

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	7	11	12	8
Total primary neoplasms	7	13	17	11
Total animals with benign neoplasms	6	8	8	7
Total benign neoplasms	6	9	11	10
Total animals with malignant neoplasms		1	2	1
Total malignant neoplasms		1	2	1
Total animals with uncertain neoplasms-benign or malignant	1	3	4	
Total uncertain neoplasms	1	3	4	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1	2	4	1
Survivors				
Terminal sacrifice	14	13	11	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation		3 (20%)	2 (13%)	1 (7%)
Inflammation, chronic active	9 (60%)	13 (87%)	12 (80%)	11 (73%)
Hepatocyte, fatty change		3 (20%)	1 (7%)	
Hepatocyte, necrosis		1 (7%)	1 (7%)	
Hepatocyte, vacuolization cytoplasmic	7 (47%)	13 (87%)	15 (100%)	15 (100%)
Mesentery	(1)	(1)	(2)	
Fat, fibrosis		1 (100%)	1 (50%)	
Fat, hemorrhage	1 (100%)	1 (100%)	2 (100%)	
Fat, inflammation, chronic active	1 (100%)	1 (100%)	2 (100%)	
Fat, necrosis	1 (100%)	1 (100%)	2 (100%)	
Stomach, forestomach	(15)	(15)	(15)	(15)
Inflammation, chronic active				1 (7%)
Epithelium, hyperkeratosis			1 (7%)	1 (7%)
Epithelium, hyperplasia	2 (13%)	4 (27%)	3 (20%)	6 (40%)
Stomach, glandular				(1)
Glands, ectasia				1 (100%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Accessory adrenal cortical nodule	1 (7%)		1 (7%)	2 (13%)
Hypertrophy	8 (53%)	9 (60%)	8 (53%)	8 (53%)
Subcapsular, hyperplasia	2 (13%)		2 (13%)	
Pituitary gland	(14)	(15)	(15)	(15)
Pars distalis, cyst	1 (7%)	1 (7%)	1 (7%)	1 (7%)
Thyroid gland	(15)	(15)	(15)	(15)
Infiltration cellular, lymphocyte		1 (7%)		
Follicle, degeneration	7 (47%)	8 (53%)	5 (33%)	5 (33%)
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Degeneration	2 (13%)	2 (13%)	2 (13%)	2 (13%)
Preputial gland		(2)	(1)	
Duct, ectasia		2 (100%)	1 (100%)	
Seminal vesicle	(1)			
Inflammation, chronic active	1 (100%)			
Testes	(15)	(15)	(15)	(15)
Cyst	1 (7%)	1 (7%)	1 (7%)	1 (7%)
Germinal epithelium, degeneration	2 (13%)	2 (13%)	1 (7%)	2 (13%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Hematopoietic System				
Lymph node, mandibular	(15)	(14)	(15)	(14)
Hyperplasia, lymphoid	1 (7%)	3 (21%)	2 (13%)	
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			2 (13%)	1 (7%)
Thymus	(15)	(15)	(14)	(14)
Atrophy	1 (7%)	3 (20%)	4 (29%)	
Cyst	1 (7%)	2 (13%)	1 (7%)	2 (14%)
Integumentary System				
Skin	(4)	(4)	(4)	(6)
Inflammation, chronic active				1 (17%)
Epidermis, hyperplasia	1 (25%)			
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation, chronic active		4 (27%)	2 (13%)	1 (7%)
Alveolar epithelium, hyperplasia			1 (7%)	1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Inflammation, chronic active		1 (7%)		
Necrosis		1 (7%)		
Nephropathy	10 (67%)	9 (60%)	12 (80%)	14 (93%)
Renal tubule, dilatation	1 (7%)			2 (13%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund		4	1	4
Natural deaths		3	1	1
Survivors				
Terminal sacrifice	15	8	13	10
Animals examined microscopically	15	15	15	15
Alimentary System				
Intestine large, rectum	(2)		(2)	
Anus, squamous cell papilloma	2 (100%)		2 (100%)	
Liver	(15)	(15)	(15)	(15)
Salivary glands	(1)			
Carcinoma	1 (100%)			
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	4 (27%)	3 (20%)	2 (13%)	4 (27%)
Squamous cell papilloma, multiple	1 (7%)	2 (13%)	3 (20%)	
Tooth	(2)	(4)	(2)	(2)
Odontogenic tumor	2 (100%)	4 (100%)	2 (100%)	2 (100%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Adrenal medulla	(15)	(15)	(15)	(15)
Pituitary gland	(15)	(14)	(15)	(15)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Hematopoietic System				
Lymph node	(1)			
Lymph node, mandibular	(15)	(14)	(15)	(15)
Lymph node, mesenteric	(15)	(13)	(15)	(15)
Spleen	(15)	(15)	(15)	(15)
Integumentary System				
Skin	(4)	(4)	(2)	(8)
Squamous cell papilloma	4 (100%)	4 (100%)	1 (50%)	4 (50%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar carcinoma		1 (7%)		1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)	1 (7%)		
Lymphoma malignant				1 (7%)
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	10	9	10
Total primary neoplasms	15	15	10	12
Total animals with benign neoplasms	8	8	8	7
Total benign neoplasms	11	10	8	9
Total animals with malignant neoplasms	2	1		1
Total malignant neoplasms	2	1		1
Total animals with uncertain neoplasms-benign or malignant	2	4	2	2
Total uncertain neoplasms	2	4	2	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund		4	1	4
Natural deaths		3	1	1
Survivors				
Terminal sacrifice	15	8	13	10
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	2 (13%)			
Inflammation, chronic active	14 (93%)	10 (67%)	12 (80%)	12 (80%)
Hepatocyte, fatty change	2 (13%)	3 (20%)		
Hepatocyte, necrosis	1 (7%)	5 (33%)	1 (7%)	3 (20%)
Hepatocyte, vacuolization cytoplasmic	6 (40%)	10 (67%)	14 (93%)	14 (93%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Epithelium, hyperkeratosis		5 (33%)	1 (7%)	1 (7%)
Epithelium, hyperplasia	2 (13%)	5 (33%)	2 (13%)	2 (13%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Accessory adrenal cortical nodule	2 (13%)	5 (33%)	4 (27%)	7 (47%)
Subcapsular, hyperplasia	10 (67%)	7 (47%)	3 (20%)	9 (60%)
Pituitary gland	(15)	(14)	(15)	(15)
Pars distalis, cyst	1 (7%)		3 (20%)	4 (27%)
Thyroid gland	(15)	(15)	(15)	(15)
Follicle, degeneration	4 (27%)	4 (27%)	4 (27%)	3 (20%)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Cyst	1 (7%)	1 (7%)	1 (7%)	2 (13%)
Inflammation, chronic active		1 (7%)		
Pigmentation, hemosiderin				1 (7%)
Uterus	(15)	(15)	(15)	(15)
Endometrium, hyperplasia, cystic	9 (60%)	8 (53%)	10 (67%)	5 (33%)
Hematopoietic System				
Lymph node, mandibular	(15)	(14)	(15)	(15)
Hyperplasia, lymphoid	3 (20%)	2 (14%)	3 (20%)	2 (13%)
Spleen	(15)	(15)	(15)	(15)
Atrophy		1 (7%)	1 (7%)	
Hematopoietic cell proliferation	1 (7%)	1 (7%)		
Necrosis				1 (7%)
Thymus	(15)	(15)	(15)	(15)
Atrophy	1 (7%)	4 (27%)	3 (20%)	3 (20%)
Cyst	4 (27%)	3 (20%)	4 (27%)	1 (7%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Integumentary System				
Skin	(4)	(4)	(2)	(8)
Hyperkeratosis			1 (50%)	
Epidermis, hyperplasia			1 (50%)	4 (50%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation, chronic active		1 (7%)		
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Inflammation, chronic active	1 (7%)			1 (7%)
Necrosis			1 (7%)	
Nephropathy	5 (33%)	6 (40%)	5 (33%)	7 (47%)
Cortex, cyst	1 (7%)			
Papilla, necrosis				1 (7%)
Renal tubule, dilatation	3 (20%)	1 (7%)	1 (7%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE B5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund sacrifice		1		
Natural death	1			
Survivors				
Terminal sacrifice	9	9	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma				1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	3 (30%)	1 (10%)	2 (20%)
Squamous cell papilloma, multiple	3 (30%)	6 (60%)	5 (50%)	5 (50%)
Tooth	(2)	(1)	(1)	(2)
Odontogenic tumor		1 (100%)	1 (100%)	1 (50%)
Odontogenic tumor, multiple	2 (100%)			1 (50%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)	(10)	(10)	(10)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)
Integumentary System				
Skin	(9)	(9)	(9)	(8)
Squamous cell papilloma		1 (11%)	1 (10%)	3 (30%)
Squamous cell papilloma, multiple	9 (100%)	8 (89%)	9 (100%)	8 (100%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma	1 (10%)		7 (70%)	3 (30%)
Alveolar/bronchiolar adenoma, multiple		2 (20%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic			1 (10%)	

TABLE B5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	9	10	9
Total primary neoplasms	17	21	24	21
Total animals with benign neoplasms	9	9	10	9
Total benign neoplasms	15	20	22	19
Animals with malignant neoplasms			1	
Total malignant neoplasms			1	
Total animals with uncertain neoplasms-benign or malignant	2	1	1	2
Total uncertain neoplasms	2	1	1	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund		1		
Natural death	1			
Survivors				
Terminal sacrifice	9	9	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)		
Inflammation, acute		1 (10%)	1 (10%)	1 (10%)
Inflammation, chronic active	9 (90%)	7 (70%)	5 (50%)	7 (70%)
Hepatocyte, necrosis	1 (10%)	1 (10%)	1 (10%)	3 (30%)
Hepatocyte, vacuolization cytoplasmic	9 (90%)	10 (100%)	9 (90%)	10 (100%)
Mesentery	(1)		(3)	(1)
Fat, fibrosis			2 (67%)	1 (100%)
Fat, inflammation, chronic active			1 (33%)	1 (100%)
Fat, necrosis	1 (100%)		3 (100%)	1 (100%)
Fat, pigmentation				1 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Epithelium, hyperplasia	3 (30%)			1 (10%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	1 (10%)	1 (10%)		1 (10%)
Atypia cellular			1 (10%)	
Hematopoietic cell proliferation	1 (10%)	1 (10%)		
Hypertrophy	8 (80%)	6 (60%)	9 (90%)	7 (70%)
Subcapsular, hyperplasia	2 (20%)	1 (10%)	1 (10%)	
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst		2 (20%)	1 (10%)	1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)
Ectopic thymus			1 (10%)	
Infiltration cellular, lymphocyte	1 (10%)	1 (10%)		
Follicle, degeneration	3 (30%)	9 (90%)	5 (50%)	4 (40%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Cyst				1 (10%)
Degeneration		1 (10%)	1 (10%)	3 (30%)
Inflammation, chronic active	1 (10%)			
Preputial gland	(2)	(4)	(2)	
Inflammation, chronic active	1 (50%)			
Duct, ectasia	2 (100%)	4 (100%)	2 (100%)	
Seminal vesicle	(1)	(1)		
Inflammation, chronic active	1 (100%)			
Inflammation, suppurative		1 (100%)		
Testes	(10)	(10)	(10)	(10)
Cyst			1 (10%)	
Mineralization				1 (10%)
Germinal epithelium, degeneration		1 (10%)	1 (10%)	3 (30%)
Interstitial cell, hyperplasia			1 (10%)	1 (10%)
Hematopoietic System				
Lymph node		(1)	(2)	
Lumbar, hematopoietic cell proliferation		1 (100%)		
Lumbar, hyperplasia, lymphoid		1 (100%)		
Mediastinal, hematopoietic cell proliferation		1 (100%)		
Mediastinal, hyperplasia, lymphoid		1 (100%)		
Mediastinal, infiltration cellular, mast cell			1 (50%)	
Mediastinal, infiltration cellular, histiocyte			1 (50%)	
Renal, hematopoietic cell proliferation		1 (100%)		
Renal, hyperplasia, lymphoid		1 (100%)		
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid		1 (10%)	1 (10%)	
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)	1 (10%)		
Necrosis	1 (10%)			
Thymus	(10)	(10)	(9)	(10)
Atrophy	2 (20%)	1 (10%)	1 (11%)	
Cyst	2 (20%)	3 (30%)	4 (44%)	3 (30%)
Ectopic parathyroid gland			2 (22%)	
Integumentary System				
Skin	(9)	(9)	(9)	(8)
Inflammation, chronic active			1 (11%)	1 (13%)
Epidermis, hyperplasia	5 (56%)	4 (44%)		1 (13%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage	1 (10%)			
Infiltration cellular, polymorphonuclear	1 (10%)			
Inflammation, chronic active	2 (20%)	1 (10%)		
Alveolar epithelium, hyperplasia		1 (10%)		

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Infarct		1 (10%)		
Inflammation, chronic active	1 (10%)			
Mineralization	1 (10%)			
Necrosis	1 (10%)			
Nephropathy	9 (90%)	8 (80%)	7 (70%)	10 (100%)
Pelvis, inflammation, chronic		1 (10%)		
Renal tubule, cyst			1 (10%)	
Renal tubule, dilatation	4 (40%)	6 (60%)	6 (60%)	5 (50%)
Renal tubule, nephropathy		1 (10%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE B7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1		1	2
Natural deaths	2	1	2	
Survivors				
Terminal sacrifice	7	9	7	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine large, rectum	(1)			
Anus, squamous cell papilloma	1 (100%)			
Liver	(10)	(10)	(10)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	5 (50%)	1 (10%)	3 (30%)	2 (20%)
Squamous cell papilloma, multiple	1 (10%)	6 (60%)	4 (40%)	4 (40%)
Tooth	(1)	(2)	(3)	(3)
Odontogenic tumor	1 (100%)	2 (100%)	3 (100%)	3 (100%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)	(10)	(10)	(10)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Oviduct		(1)		
Histiocytic sarcoma		1 (100%)		
Hematopoietic System				
Lymph node		(1)		(1)
Spleen	(10)	(10)	(10)	(10)
Thymus	(10)	(10)	(9)	(10)
Integumentary System				
Skin	(6)	(7)	(7)	(7)
Squamous cell papilloma	1 (17%)	2 (29%)	4 (57%)	4 (57%)
Squamous cell papilloma, multiple	5 (83%)	5 (71%)	2 (29%)	3 (43%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma				2 (20%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)

TABLE B7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic	3 (30%)		1 (10%)	1 (10%)
Lymphoma malignant		1 (10%)		
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	9	8	9
Total primary neoplasms	17	18	17	19
Total animals with benign neoplasms	7	8	7	8
Total benign neoplasms	13	15	13	15
Total animals with malignant neoplasms	3	1	1	1
Total malignant neoplasms	3	1	1	1
Total animals with uncertain neoplasms-benign or malignant	1	2	3	3
Total uncertain neoplasms	1	2	3	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1		1	2
Natural deaths	2	1	2	
Survivors				
Terminal sacrifice	7	9	7	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Inflammation, chronic active	7 (70%)	10 (100%)	9 (90%)	8 (80%)
Hepatocyte, fatty change				1 (10%)
Hepatocyte, necrosis	1 (10%)	1 (10%)	1 (10%)	4 (40%)
Hepatocyte, vacuolization cytoplasmic	7 (70%)	9 (90%)	9 (90%)	10 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Epithelium, hyperkeratosis		1 (10%)		
Epithelium, hyperplasia		1 (10%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	2 (20%)	2 (20%)	2 (20%)	3 (30%)
Subcapsular, hyperplasia	3 (30%)	5 (50%)	4 (40%)	8 (80%)
Adrenal medulla	(10)	(10)	(10)	(10)
Mineralization			1 (10%)	
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst	2 (20%)	2 (20%)	2 (20%)	2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Ectopic thymus				1 (10%)
Follicle, degeneration	3 (30%)	6 (60%)	5 (50%)	5 (50%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Cyst		7 (70%)	4 (40%)	1 (10%)
Inflammation, chronic active		1 (10%)		
Uterus	(10)	(10)	(10)	(10)
Endometrium, hyperplasia, cystic	7 (70%)	9 (90%)	7 (70%)	7 (70%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid		1 (10%)	1 (10%)	1 (10%)
Spleen	(10)	(10)	(10)	(10)
Atrophy		1 (10%)		
Hematopoietic cell proliferation		1 (10%)		1 (10%)
Thymus	(10)	(10)	(9)	(10)
Atrophy	3 (30%)	1 (10%)	1 (11%)	1 (10%)
Cyst	3 (30%)	4 (40%)	2 (22%)	5 (50%)
Ectopic parathyroid gland	1 (10%)			
Thrombosis	1 (10%)			
Thymocyte, necrosis				1 (10%)
Integumentary System				
Skin	(6)	(7)	(7)	(7)
Hyperkeratosis			1 (14%)	
Inflammation, chronic active			1 (14%)	
Epidermis, hyperplasia	1 (17%)		2 (29%)	
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Infarct			1 (10%)	
Mineralization			1 (10%)	
Nephropathy	3 (30%)	5 (50%)	2 (20%)	7 (70%)
Glomerulus, inflammation, membranoproliferative	3 (30%)		1 (10%)	1 (10%)
Renal tubule, dilatation	3 (30%)	1 (10%)	2 (20%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Respiratory
Special Senses System

APPENDIX C
SUMMARY OF LESIONS IN p53 HAPLOINSUFFICIENT MICE IN THE DRINKING WATER STUDIES OF DICHLOROACETIC ACID

TABLE C1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE C2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	1 (7%)			
Inflammation, chronic active	15 (100%)	13 (87%)	15 (100%)	13 (87%)
Hepatocyte, fatty change	10 (67%)	2 (13%)	1 (7%)	
Hepatocyte, necrosis	1 (7%)		1 (7%)	
Hepatocyte, vacuolization cytoplasmic	15 (100%)	15 (100%)	15 (100%)	15 (100%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Accessory adrenal cortical nodule	1 (7%)		1 (7%)	
Hypertrophy	1 (7%)		1 (7%)	2 (13%)
Subcapsular, hyperplasia	4 (27%)		3 (20%)	3 (20%)
Pituitary gland	(15)	(15)	(15)	(15)
Pars distalis, cyst	3 (20%)	2 (13%)	2 (13%)	1 (7%)
Pars distalis, hyperplasia			6 (40%)	
Thyroid gland	(15)	(15)	(15)	(15)
Ectopic thymus				2 (13%)
Follicle, cyst			1 (7%)	
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Granuloma sperm	1 (7%)			
Infiltration cellular, lymphocyte			3 (20%)	
Inflammation, chronic active			1 (7%)	
Testes	(15)	(15)	(15)	(15)
Mineralization	1 (7%)		1 (7%)	
Germinal epithelium, degeneration	2 (13%)	4 (27%)	3 (20%)	1 (7%)
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(15)
Hyperplasia, lymphoid			2 (13%)	1 (7%)
Lymph node, mesenteric	(15)	(15)	(15)	(15)
Hyperplasia, lymphoid	1 (7%)	1 (7%)		
Thymus	(15)	(15)	(15)	(15)
Cyst	9 (60%)	9 (60%)	9 (60%)	9 (60%)
Ectopic parathyroid gland			1 (7%)	
Hyperplasia, lymphoid		1 (7%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Mineralization			1 (7%)	
Nephropathy	8 (53%)	10 (67%)	11 (73%)	12 (80%)
Renal tube, dilatation	2 (13%)	4 (27%)		2 (13%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

TABLE C3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund				1
Natural death			1	
Survivors				
Terminal sacrifice	15	15	14	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Intestine large, cecum	(15)	(15)	(15)	(15)
Liver	(15)	(15)	(15)	(15)
Mesentery				(1)
Endocrine System				
Pituitary gland	(15)	(15)	(15)	(15)
Pars distalis, adenoma		1 (7%)		
Genital System				
Ovary	(15)	(15)	(15)	(15)
Uterus	(15)	(15)	(15)	(15)
Polyp stromal	1 (7%)			
Hematopoietic System				
Lymph node, mesenteric	(15)	(15)	(15)	(15)
Thymus	(15)	(15)	(15)	(15)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Lymphoma malignant			1 (7%)	1 (7%)

Systems Examined with No Neoplasms Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE C3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	1	1	1	1
Total primary neoplasms	1	1	1	1
Total animals with benign neoplasms	1	1		
Total benign neoplasms	1	1		
Total animals with malignant neoplasms			1	1
Total malignant neoplasms			1	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund				1
Natural death			1	
Survivors				
Terminal sacrifice	15	15	14	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Inflammation, chronic active	15 (100%)	14 (93%)	13 (87%)	14 (93%)
Hepatocyte, necrosis	1 (7%)			
Hepatocyte, vacuolization cytoplasmic	3 (20%)	15 (100%)	15 (100%)	15 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Inflammation, chronic active				1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Accessory adrenal cortical nodule	4 (27%)	2 (13%)	1 (7%)	
Subcapsular, hyperplasia	14 (93%)	14 (93%)	15 (100%)	14 (93%)
Parathyroid gland	(1)			
Cyst	1 (100%)			
Pituitary gland	(15)	(15)	(15)	(15)
Pars distalis, cyst	1 (7%)	2 (13%)	3 (20%)	
Pars distalis, hyperplasia	1 (7%)		3 (20%)	5 (33%)
Thyroid gland	(15)	(15)	(15)	(15)
Ectopic thymus	1 (7%)			
Infiltration cellular, lymphocyte	2 (13%)	1 (7%)		
Follicle, degeneration	1 (7%)	3 (20%)		
Genital System				
Ovary	(15)	(15)	(15)	(15)
Cyst		2 (13%)	1 (7%)	1 (7%)
Uterus	(15)	(15)	(15)	(15)
Inflammation, chronic active	1 (7%)			1 (7%)
Endometrium, hyperplasia, cystic	8 (53%)	12 (80%)	13 (87%)	13 (87%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Hematopoietic System				
Lymph node		(1)		
Mediastinal, hyperplasia, lymphoid		1 (100%)		
Lymph node, mandibular	(15)	(15)	(15)	(15)
Hyperplasia, lymphoid	1 (7%)		4 (27%)	4 (27%)
Lymph node, mesenteric	(15)	(15)	(15)	(15)
Hyperplasia, lymphoid				1 (7%)
Spleen	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Thymus	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Cyst	10 (67%)	11 (73%)	11 (73%)	12 (80%)
Ectopic parathyroid gland	2 (13%)	2 (13%)	1 (7%)	2 (13%)
Thymocyte, necrosis		6 (40%)	2 (13%)	1 (7%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation, chronic active		2 (13%)	3 (20%)	1 (7%)
Alveolus, infiltration cellular, histiocyte		1 (7%)		
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Cyst				1 (7%)
Mineralization			4 (27%)	1 (7%)
Nephropathy	3 (20%)	11 (73%)	7 (47%)	9 (60%)
Renal tubule, dilatation			2 (13%)	1 (7%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE C5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Natural deaths	1		1	
Survivors				
Terminal sacrifice	9	10	9	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma		2 (20%)		
Osteosarcoma, metastatic, bone	1 (10%)			
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Thymoma malignant, metastatic, thymus		1 (10%)		
Hematopoietic System				
Spleen	(10)	(10)	(10)	(10)
Thymus	(10)	(10)	(10)	(10)
Thymoma malignant		1 (10%)		
Musculoskeletal System				
Bone	(1)	(1)		
Osteosarcoma	1 (100%)	1 (100%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Thymoma malignant, metastatic, thymus		1 (10%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Thymoma malignant, metastatic, thymus		1 (10%)		
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Lymphoma malignant			1 (10%)	

TABLE C5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Integumentary System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	1	3	1	
Total primary neoplasms	1	4	1	
Total animals with benign neoplasms		2		
Total benign neoplasms		2		
Total animals with malignant neoplasms	1	2	1	
Total malignant neoplasms	1	2	1	
Total animals with metastatic neoplasms	1	1		
Total metastatic neoplasms	1	3		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Natural deaths	1		1	
Survivors				
Terminal sacrifice	9	10	9	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)			
Inflammation, chronic active	9 (90%)	8 (80%)	7 (70%)	9 (90%)
Hepatocyte, fatty change	6 (60%)	5 (50%)		
Hepatocyte, necrosis	1 (10%)			
Hepatocyte, vacuolization cytoplasmic	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Inflammation, chronic active	1 (10%)		1 (10%)	
Ulcer	1 (10%)		1 (10%)	
Epithelium, hyperkeratosis	1 (10%)		1 (10%)	
Epithelium, hyperplasia	1 (10%)		1 (10%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	3 (30%)	3 (30%)	1 (10%)	
Subcapsular, hyperplasia	3 (30%)	5 (50%)	3 (30%)	3 (30%)
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst	4 (40%)	2 (20%)	2 (20%)	
Thyroid gland	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)	1 (10%)		
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Degeneration				1 (10%)
Inflammation, chronic active	3 (30%)	2 (20%)	1 (10%)	3 (30%)
Testes	(10)	(10)	(10)	(10)
Mineralization				1 (10%)
Germinal epithelium, degeneration	5 (50%)	4 (40%)	2 (20%)	4 (40%)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid	1 (10%)			
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)	1 (10%)		
Thymus	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			
Cyst	6 (60%)	3 (30%)	2 (20%)	2 (20%)
Ectopic parathyroid gland			2 (20%)	3 (30%)
Hyperplasia, lymphoid	1 (10%)	1 (10%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Thrombosis		1 (10%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Hydronephrosis				1 (10%)
Metaplasia, osseous				1 (10%)
Mineralization	1 (10%)			
Nephropathy	9 (90%)	6 (60%)	8 (80%)	8 (80%)
Renal tubule, dilatation	1 (10%)	2 (20%)		1 (10%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE C7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund		1		
Natural death				1
Survivors				
Terminal sacrifice	10	9	10	9
Animals examined microscopically	10	10	10	10
Genital System				
Uterus	(10)	(10)	(10)	(10)
Polyp stromal		1 (10%)		
Integumentary System				
Skin		(1)		(1)
Subcutaneous tissue, rhabdomyosarcoma				1 (100%)
Musculoskeletal System				
Bone		(1)		(1)
Osteosarcoma		1 (100%)		1 (100%)
Skeletal muscle		(1)		(1)
Osteosarcoma, metastatic, bone		1 (100%)		1 (100%)
Nervous System				
Spinal cord		(1)		
Osteosarcoma, metastatic, bone		1 (100%)		

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Respiratory System
Special Senses System
Urinary System

TABLE C7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[b]	2		2	
Total primary neoplasms	2		2	
Total animals with benign neoplasms	1			
Total benign neoplasms	1			
Total animals with malignant neoplasms	1		2	
Total malignant neoplasms	1		2	
Total animals with metastatic neoplasms	1		1	
Total metastatic neoplasms	2		1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund		1		
Natural death				1
Survivors				
Terminal sacrifice	10	9	10	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Inflammation, chronic active	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Hepatocyte, fatty change	1 (10%)			
Hepatocyte, necrosis			1 (10%)	
Hepatocyte, vacuolization cytoplasmic	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule		2 (20%)		
Subcapsular, hyperplasia	10 (100%)	10 (100%)	9 (90%)	10 (100%)
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst			1 (10%)	1 (10%)
Pars distalis, hyperplasia		1 (10%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Ectopic thymus				1 (10%)
Infiltration cellular, lymphocyte				2 (20%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Cyst		1 (10%)	1 (10%)	7 (70%)
Uterus	(10)	(10)	(10)	(10)
Inflammation, chronic active	2 (20%)	1 (10%)	1 (10%)	
Endometrium, hyperplasia, cystic	7 (70%)	9 (90%)	9 (90%)	9 (90%)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid		2 (20%)		
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)	2 (20%)		2 (20%)
Hyperplasia, lymphoid		1 (10%)		
Thymus	(10)	(10)	(10)	(10)
Atrophy				1 (10%)
Cyst	9 (90%)	8 (80%)	7 (70%)	8 (80%)
Ectopic parathyroid gland		1 (10%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Integumentary System				
Skin		(1)		(1)
Hyperkeratosis		1 (100%)		
Hyperplasia		1 (100%)		
Inflammation, chronic active		1 (100%)		
Ulcer		1 (100%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Atrophy				1 (10%)
Metaplasia, osseous			1 (10%)	
Mineralization		2 (20%)	3 (30%)	
Nephropathy	4 (40%)	4 (40%)	7 (70%)	5 (50%)
Renal tubule, dilatation			1 (10%)	1 (10%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

APPENDIX D
GENETIC TOXICOLOGY

TABLE D1
Mutagenicity of Dichloroacetic Acid in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+30% hamster S9	+30% rat S9
TA100[c]	0	129 ± 2.9	97 ± 8.8	109 ± 13.4	121 ± 8.6	156 ± 4.3
	33	134 ± 7.2			124 ± 10.2	147 ± 3.2
	100	131 ± 11.7	104 ± 10.4		135 ± 5.8	162 ± 5.0
	333	153 ± 0.7	111 ± 7.4	96 ± 1.9	140 ± 6.4	167 ± 5.0
	666		120 ± 3.3	146 ± 13.9		
	1,000	188 ± 1.5	129 ± 9.3	157 ± 11.2	133 ± 11.4	175 ± 0.9
	1,666		170 ± 4.4	158 ± 6.0		
	3,333	307 ± 14.6	186 ± 4.6	238 ± 4.0	135 ± 3.5	160 ± 2.5
	6,666			Toxic		
Trial summary		Positive	Positive	Positive	Negative	Negative
Positive control[d]		734 ± 20.6	590 ± 24.4	309 ± 18.9	542 ± 22.7	373 ± 1.2
TA100[e]	0	132 ± 3.3	105 ± 12.0		142 ± 15.4	149 ± 1.7
	33	113 ± 13.0			153 ± 3.8	143 ± 4.8
	100	128 ± 5.0			161 ± 1.8	164 ± 11.7
	333	135 ± 9.0	113 ± 8.3		133 ± 3.8	148 ± 7.0
	666		131 ± 5.2			
	1,000	180 ± 3.2	158 ± 5.5		145 ± 9.9	148 ± 3.8
	1,666		194 ± 5.0			
	3,333	442 ± 19.6	315 ± 30.7		139 ± 2.2	137 ± 3.0
	6,666		Toxic			
Trial summary		Positive	Positive		Negative	Negative
Positive control		951 ± 25.6	919 ± 17.2		1,022 ± 22.5	629 ± 50.7
TA1535[c]	0	12 ± 2.0	9 ± 1.7	9 ± 0.6		
	333	14 ± 1.7				
	666	10 ± 0.3	7 ± 2.3	17 ± 0.7		
	1,000	15 ± 2.4	10 ± 2.3	14 ± 2.7		
	1,666		12 ± 1.0	16 ± 3.5		
	3,333	38 ± 5.6	19 ± 2.3	42 ± 1.9		
	6,666	78 ± 7.2	19 ± 8.4	0 ± 0.0		
Trial summary		Positive	Weakly Positive	Equivocal		
Positive control		334 ± 23.2	243 ± 4.9	307 ± 43.6		

TABLE D1
Mutagenicity of Dichloroacetic Acid in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+30% hamster S9	+30% rat S9
TA98[c]	0	19 ± 2.3	29 ± 1.8	31 ± 3.2	20 ± 0.9
	3		27 ± 1.5		
	10		22 ± 1.8		
	33	27 ± 4.5	22 ± 3.0	26 ± 2.9	20 ± 2.3
	100	29 ± 4.9	20 ± 3.0	31 ± 3.8	26 ± 2.3
	333	30 ± 0.9	24 ± 4.8	22 ± 2.4	20 ± 0.3
	1,000	32 ± 2.3		25 ± 3.2	22 ± 2.9
	3,333	32 ± 5.2		17 ± 8.7	19 ± 2.2
Trial summary		Equivocal	Negative	Negative	Negative
Positive control		527 ± 18.6	476 ± 20.2	486 ± 11.4	121 ± 7.4
TA98[e]	0	17 ± 0.3		22 ± 2.1	26 ± 1.5
	33	19 ± 1.2		20 ± 2.4	17 ± 1.7
	100	18 ± 2.0		26 ± 0.9	20 ± 3.2
	333	19 ± 2.6		25 ± 2.5	12 ± 0.3
	1,000	18 ± 1.5		21 ± 2.5	18 ± 2.0
	3,333	11 ± 5.2		23 ± 3.2	25 ± 2.3
Trial summary		Negative		Negative	Negative
Positive control		319 ± 7.7		841 ± 75.9	511 ± 34.1

[a] The study was performed at SRI International. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Dimethylsulfoxide was the solvent.
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Water was the solvent.

TABLE D2
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Dermal Administration of Dichloroacetic Acid for 26 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Water:Acetone[d]	0	13	1.35 ± 0.18		2.8 ± 0.1
Dichloroacetic Acid	31.25	14	1.25 ± 0.24	0.6218	2.9 ± 0.2
	125	14	1.18 ± 0.16	0.7084	2.8 ± 0.2
	500	12	0.71 ± 0.16	0.9865	3.2 ± 0.2
			P=0.989[e]		
Female					
Water:Acetone	0	11	1.14 ± 0.20		2.6 ± 0.2
Dichloroacetic Acid	31.25	12	1.58 ± 0.17	0.0977	3.1 ± 0.2
	125	14	1.25 ± 0.23	0.3578	3.2 ± 0.2
	500	15	1.07 ± 0.15	0.5938	3.6 ± 0.2
			P=0.857		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
[b] PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control group; significant at P≤0.008 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE D3
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Administration of Dichloroacetic Acid in Drinking Water for 26 Weeks[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Water[d]	0	14	1.43 ± 0.21		3.1 ± 0.1
Dichloroacetic Acid	500	13	0.96 ± 0.20	0.9411	3.4 ± 0.4
	1,000	11	1.50 ± 0.32	0.4178	2.8 ± 0.2
	2,000	14	1.39 ± 0.21	0.5448	2.9 ± 0.2
			P=0.343[e]		
Female					
Water	0	15	1.17 ± 0.15		3.7 ± 0.5
Dichloroacetic Acid	500	8	1.13 ± 0.28	0.5499	3.3 ± 0.4
	1,000	13	1.35 ± 0.20	0.2744	3.1 ± 0.1
	2,000	10	1.25 ± 0.17	0.3960	3.1 ± 0.2
			P=0.346		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.008 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE D4
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of p53 Haploinsufficient Mice Following Administration of Dichloroacetic Acid in Drinking Water for 26 Weeks[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Water[d]	0	15	1.60 ± 0.20		2.7 ± 0.1
Dichloroacetic Acid	500	15	1.90 ± 0.20	0.1897	2.8 ± 0.2
	1,000	15	1.53 ± 0.19	0.5818	2.7 ± 0.1
	2,000	15	1.80 ± 0.24	0.2761	2.6 ± 0.1
			P=0.384[e]		
Female					
Water	0	15	1.00 ± 0.22		3.0 ± 0.2
Dichloroacetic Acid	500	15	0.83 ± 0.12	0.7500	2.6 ± 0.1
	1,000	14	1.04 ± 0.22	0.4464	2.4 ± 0.1
	2,000	14	1.18 ± 0.21	0.2571	2.4 ± 0.1
			P=0.169		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
[b] PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the untreated control group; significant at P≤0.008 (ILS, 1990)
[e] Untreated control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE D5
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of B6C3F$_1$ Mice Following Administration of Dichloroacetic Acid in Drinking Water for 3 Months[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Water[d]	0	10	2.30 ± 0.40		3.0 ± 0.1
Dichloroacetic Acid	67	10	2.10 ± 0.43	0.6186	2.6 ± 0.1
	125	10	2.70 ± 0.42	0.2856	2.4 ± 0.1
	250	10	2.90 ± 0.43	0.2024	2.5 ± 0.1
	500	10	2.60 ± 0.31	0.3339	2.4 ± 0.1
	1,000	10	1.60 ± 0.40	0.8691	2.3 ± 0.1
			P=0.887[e]		
Female					
Water	0	10	1.80 ± 0.42		2.3 ± 0.1
Dichloroacetic Acid	67	10	2.00 ± 0.33	0.3727	2.5 ± 0.1
	125	10	2.30 ± 0.45	0.2172	2.5 ± 0.1
	250	10	2.00 ± 0.21	0.3727	2.6 ± 0.1
	500	10	2.60 ± 0.50	0.1136	2.5 ± 0.1
	1,000	10	3.30 ± 0.52	0.0177	2.5 ± 0.1
			P=0.007		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.005 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX E
HEMATOLOGY RESULTS

TABLE E1
Hematology Data for Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
n	13	14	14	11
Hematocrit (%)	41.8 ± 0.5	42.4 ± 0.6	41.3 ± 0.6	40.9 ± 0.5
Hemoglobin (g/dL)	13.8 ± 0.2	13.9 ± 0.2	13.6 ± 0.2	13.3 ± 0.2
Erythrocytes (10^6/μL)	9.63 ± 0.15	9.77 ± 0.17	9.41 ± 0.16	9.50 ± 0.16
Reticulocytes (10^6/μL)	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	43.4 ± 0.3	43.4 ± 0.3	43.9 ± 0.3	43.1 ± 0.4
Mean cell hemoglobin (pg)	14.3 ± 0.1	14.3 ± 0.1	14.5 ± 0.1	14.0 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.1	32.8 ± 0.1	32.9 ± 0.2	32.4 ± 0.1*
Platelets (10^3/μL)	975.3 ± 18.5	1,010.4 ± 20.2	1,050.7 ± 36.9*	1,058.3 ± 22.5**
Leukocytes (10^3/μL)	3.08 ± 0.19	2.94 ± 0.33	2.32 ± 0.21	3.17 ± 0.44
Segmented neutrophils (10^3/μL)	0.44 ± 0.05	0.40 ± 0.08	0.36 ± 0.06	0.71 ± 0.21
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.61 ± 0.16	2.50 ± 0.25	1.93 ± 0.16	2.43 ± 0.37
Monocytes (10^3/μL)	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Female				
n	10	12	14	13
Hematocrit (%)	43.2 ± 0.7	44.4 ± 0.5	44.0 ± 0.6	42.4 ± 0.4
Hemoglobin (g/dL)	14.2 ± 0.2	14.7 ± 0.2	14.4 ± 0.2	13.9 ± 0.1
Erythrocytes (10^6/μL)	9.64 ± 0.19	9.94 ± 0.21	9.81 ± 0.14	9.58 ± 0.12
Reticulocytes (10^6/μL)	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.9 ± 0.5	44.7 ± 0.4	44.9 ± 0.2	44.3 ± 0.2
Mean cell hemoglobin (pg)	14.8 ± 0.2	14.8 ± 0.2	14.7 ± 0.1	14.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.1	33.2 ± 0.2	32.7 ± 0.1	32.8 ± 0.1
Platelets (10^3/μL)	893.3 ± 75.2	770.0 ± 25.8	874.9 ± 40.3	927.6 ± 32.8
Leukocytes (10^3/μL)	3.94 ± 0.41	2.97 ± 0.18	3.74 ± 0.31	4.22 ± 0.22
Segmented neutrophils (10^3/μL)	1.15 ± 0.43	0.28 ± 0.05*	0.57 ± 0.14	0.47 ± 0.07
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.71 ± 0.26	2.64 ± 0.13	3.13 ± 0.22	3.66 ± 0.19**
Monocytes (10^3/μL)	0.07 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.07 ± 0.03
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE E2
Hematology Data for Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	14	13	11	14
Hematocrit (%)	42.4 ± 0.3	43.1 ± 0.7	43.5 ± 0.8	44.3 ± 0.5*
Hemoglobin (g/dL)	14.1 ± 0.1	14.4 ± 0.2	14.5 ± 0.3	14.6 ± 0.2
Erythrocytes (10^6/μL)	9.82 ± 0.08	10.29 ± 0.29	10.35 ± 0.30	10.33 ± 0.15
Reticulocytes (10^6/μL)	0.17 ± 0.01	0.18 ± 0.02	0.15 ± 0.01	0.14 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	43.2 ± 0.1	42.1 ± 0.6	42.2 ± 0.7	42.9 ± 0.2
Mean cell hemoglobin (pg)	14.4 ± 0.1	14.1 ± 0.2	14.1 ± 0.3	14.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.4 ± 0.2	33.4 ± 0.3	33.0 ± 0.1
Platelets (10^3/μL)	1,119.8 ± 18.5	1,326.5 ± 83.4	1,201.4 ± 76.8	1,112.0 ± 25.9
Leukocytes (10^3/μL)	3.14 ± 0.19	4.60 ± 0.52	4.85 ± 0.45**	3.56 ± 0.38
Segmented neutrophils (10^3/μL)	0.59 ± 0.07	1.52 ± 0.43*	1.26 ± 0.18**	0.66 ± 0.10
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.54 ± 0.18	3.03 ± 0.13	3.55 ± 0.28*	2.88 ± 0.31
Monocytes (10^3/μL)	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Female				
n	13	8	13	10
Hematocrit (%)	43.5 ± 0.5	44.1 ± 0.4	43.7 ± 0.3	44.1 ± 0.9
Hemoglobin (g/dL)	14.4 ± 0.1	14.5 ± 0.1	14.4 ± 0.1	14.6 ± 0.3
Erythrocytes (10^6/μL)	9.70 ± 0.15	10.13 ± 0.19	9.71 ± 0.15	10.07 ± 0.29
Reticulocytes (10^6/μL)	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.14 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.9 ± 0.3	43.6 ± 0.8	45.1 ± 0.4	43.9 ± 0.6
Mean cell hemoglobin (pg)	14.9 ± 0.1	14.4 ± 0.3	14.9 ± 0.2	14.6 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.1	33.0 ± 0.1	33.0 ± 0.1	33.2 ± 0.1
Platelets (10^3/μL)	917.0 ± 25.9	1,071.8 ± 93.3	904.7 ± 40.9	881.3 ± 30.4
Leukocytes (10^3/μL)	3.97 ± 0.22	4.59 ± 0.35	4.15 ± 0.25	4.28 ± 0.42
Segmented neutrophils (10^3/μL)	0.57 ± 0.07	0.79 ± 0.14	0.63 ± 0.09	0.68 ± 0.12
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	3.39 ± 0.17	3.76 ± 0.26	3.50 ± 0.19	3.57 ± 0.36
Monocytes (10^3/μL)	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

* Significantly different ($P \le 0.05$) from the control group by Dunn's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE E3
Hematology Data for p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	15	15	15	15
Hematocrit (%)	47.7 ± 0.5	47.2 ± 0.4	46.8 ± 0.4	47.3 ± 0.5
Hemoglobin (g/dL)	15.6 ± 0.2	15.5 ± 0.1	15.4 ± 0.1	15.5 ± 0.2
Erythrocytes (10^6/μL)	10.70 ± 0.13	10.73 ± 0.10	10.71 ± 0.09	10.84 ± 0.15
Reticulocytes (10^6/μL)	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.6 ± 0.2	44.0 ± 0.2*	43.6 ± 0.2**	43.6 ± 0.2**
Mean cell hemoglobin (pg)	14.6 ± 0.1	14.5 ± 0.1	14.4 ± 0.0	14.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.1	33.0 ± 0.1	33.1 ± 0.1*	32.8 ± 0.1
Platelets (10^3/μL)	939.4 ± 25.8	970.5 ± 24.8	1,122.7 ± 75.0**	1,074.8 ± 48.3**
Leukocytes (10^3/μL)	6.28 ± 0.40	5.75 ± 0.34	6.43 ± 0.39	6.12 ± 0.52
Segmented neutrophils (10^3/μL)	0.88 ± 0.08	0.73 ± 0.06	1.09 ± 0.14	1.01 ± 0.14
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	5.34 ± 0.33	4.96 ± 0.29	5.26 ± 0.33	5.03 ± 0.39
Monocytes (10^3/μL)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
Female				
n	15	15	14	14
Hematocrit (%)	45.6 ± 0.4	45.9 ± 0.5	45.5 ± 0.3	45.0 ± 0.4
Hemoglobin (g/dL)	15.2 ± 0.1	15.2 ± 0.2	15.2 ± 0.1	15.0 ± 0.1
Erythrocytes (10^6/μL)	10.31 ± 0.12	10.35 ± 0.14	10.35 ± 0.07	10.51 ± 0.09
Reticulocytes (10^6/μL)	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.3 ± 0.2	44.3 ± 0.2	44.0 ± 0.2	42.8 ± 0.2**
Mean cell hemoglobin (pg)	14.8 ± 0.1	14.7 ± 0.1	14.6 ± 0.1	14.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.1 ± 0.1	33.3 ± 0.2	33.4 ± 0.2
Platelets (10^3/μL)	831.9 ± 20.9	871.4 ± 48.6	964.4 ± 43.6*	867.1 ± 37.6
Leukocytes (10^3/μL)	2.91 ± 0.17	3.55 ± 0.37	3.03 ± 0.20	4.33 ± 0.28**
Segmented neutrophils (10^3/μL)	0.32 ± 0.02	0.51 ± 0.25	0.49 ± 0.06*	0.75 ± 0.08**
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.55 ± 0.16	3.00 ± 0.27	2.47 ± 0.17	3.50 ± 0.21*
Monocytes (10^3/μL)	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.02

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX F
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
n	13	14	14	12
Necropsy body wt	33.6 ± 0.9	33.3 ± 0.6	33.6 ± 0.8	33.3 ± 1.2
Heart				
Absolute	0.176 ± 0.005	0.175 ± 0.003	0.182 ± 0.007	0.175 ± 0.006
Relative	5.242 ± 0.112	5.269 ± 0.093	5.421 ± 0.183	5.265 ± 0.123
R. Kidney				
Absolute	0.299 ± 0.007[b]	0.308 ± 0.007	0.315 ± 0.008	0.300 ± 0.010
Relative	8.911 ± 0.236[b]	9.280 ± 0.230	9.429 ± 0.282	9.064 ± 0.322
Liver				
Absolute	1.586 ± 0.046	1.580 ± 0.030	1.856 ± 0.061**	2.642 ± 0.103**
Relative	47.221 ± 0.601	47.558 ± 0.667	55.113 ± 0.998**	79.321 ± 1.631**
Lung				
Absolute	0.279 ± 0.013	0.268 ± 0.012	0.278 ± 0.013	0.266 ± 0.015
Relative	8.298 ± 0.272	8.080 ± 0.366	8.309 ± 0.385	8.105 ± 0.527
R. Testis				
Absolute	0.086 ± 0.004	0.086 ± 0.003	0.092 ± 0.003	0.081 ± 0.002
Relative	2.563 ± 0.113	2.591 ± 0.076	2.743 ± 0.100	2.471 ± 0.102
Thymus				
Absolute	0.030 ± 0.002	0.033 ± 0.002	0.028 ± 0.002	0.029 ± 0.003
Relative	0.906 ± 0.068	0.998 ± 0.044	0.835 ± 0.051	0.842 ± 0.083
Female				
n	11	12	14	15
Necropsy body wt	25.4 ± 1.3	27.3 ± 1.0	27.5 ± 1.4	27.0 ± 0.5
Heart				
Absolute	0.139 ± 0.006	0.135 ± 0.002	0.150 ± 0.004	0.144 ± 0.004
Relative	5.531 ± 0.178	5.012 ± 0.146*	5.521 ± 0.141	5.323 ± 0.107
R. Kidney				
Absolute	0.208 ± 0.007	0.207 ± 0.004	0.207 ± 0.005	0.206 ± 0.006
Relative	8.284 ± 0.268	7.674 ± 0.207	7.661 ± 0.250	7.658 ± 0.269
Liver				
Absolute	1.344 ± 0.073	1.346 ± 0.041	1.516 ± 0.056*	2.241 ± 0.042**
Relative	52.997 ± 1.477	49.543 ± 0.645	55.501 ± 1.165	83.136 ± 1.130**
Lung				
Absolute	0.237 ± 0.014[c]	0.265 ± 0.014	0.278 ± 0.012	0.241 ± 0.011
Relative	9.634 ± 0.684[c]	9.845 ± 0.595	10.206 ± 0.416	8.931 ± 0.405
Thymus				
Absolute	0.028 ± 0.003	0.034 ± 0.002	0.031 ± 0.002	0.032 ± 0.001
Relative	1.096 ± 0.067	1.229 ± 0.062	1.137 ± 0.066	1.175 ± 0.045

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).
[b] n=12
[c] n=10

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Dichloroacetic Acid[a]

	Vehicle Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Male				
n	9	6	8	7
Necropsy body wt	37.2 ± 1.5	34.0 ± 0.8	36.7 ± 1.1	34.0 ± 1.1
Heart				
Absolute	0.199 ± 0.007	0.225 ± 0.004	0.199 ± 0.009	0.187 ± 0.008
Relative	5.413 ± 0.266	6.640 ± 0.167**	5.411 ± 0.214	5.518 ± 0.191
R. Kidney				
Absolute	0.392 ± 0.023	0.329 ± 0.006*	0.350 ± 0.010	0.354 ± 0.013
Relative	10.652 ± 0.752	9.698 ± 0.177	9.562 ± 0.302	10.431 ± 0.237
Liver				
Absolute	1.985 ± 0.072	1.907 ± 0.048	2.311 ± 0.062**	3.169 ± 0.130**
Relative	53.630 ± 1.356	56.197 ± 1.729	63.108 ± 1.597**	93.239 ± 1.904**
Lung				
Absolute	0.243 ± 0.013	0.306 ± 0.029	0.270 ± 0.023[b]	0.256 ± 0.024
Relative	6.625 ± 0.450	8.987 ± 0.865*	7.428 ± 0.688[b]	7.504 ± 0.615
R. Testis				
Absolute	0.084 ± 0.005	0.088 ± 0.003	0.087 ± 0.004	0.070 ± 0.002
Relative	2.278 ± 0.153	2.579 ± 0.101	2.378 ± 0.140	2.071 ± 0.092
Thymus				
Absolute	0.032 ± 0.003	0.032 ± 0.003	0.027 ± 0.002	0.020 ± 0.003**
Relative	0.856 ± 0.079	0.929 ± 0.075	0.738 ± 0.064	0.581 ± 0.077*
Female				
n	8	5	6	8
Necropsy body wt	27.3 ± 0.9	28.8 ± 1.6	29.9 ± 1.3[b]	35.4 ± 3.6*
Heart				
Absolute	0.166 ± 0.012	0.154 ± 0.009	0.156 ± 0.009	0.157 ± 0.008
Relative	6.048 ± 0.318	5.403 ± 0.441	5.427 ± 0.322	4.595 ± 0.248**
R. Kidney				
Absolute	0.238 ± 0.004	0.226 ± 0.011	0.233 ± 0.012	0.261 ± 0.018
Relative	8.756 ± 0.285	7.865 ± 0.343	8.091 ± 0.293	7.749 ± 0.674
Liver				
Absolute	1.615 ± 0.044	1.637 ± 0.109	1.916 ± 0.117	3.015 ± 0.158**
Relative	59.456 ± 1.946	56.900 ± 2.579	66.450 ± 3.486	88.320 ± 4.861**
Lung				
Absolute	0.245 ± 0.023	0.236 ± 0.019	0.233 ± 0.019	0.227 ± 0.017
Relative	8.988 ± 0.774	8.413 ± 1.087	8.120 ± 0.683	6.670 ± 0.583
Thymus				
Absolute	0.020 ± 0.002	0.026 ± 0.001	0.031 ± 0.002*	0.034 ± 0.005**
Relative	0.744 ± 0.078	0.918 ± 0.063	1.065 ± 0.063*	0.963 ± 0.106

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).
[b] n=7

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	14	13	11	14
Necropsy body wt	34.5 ± 0.8	35.6 ± 1.9	35.9 ± 1.3	34.1 ± 0.7
Heart				
Absolute	0.187 ± 0.004	0.185 ± 0.008	0.179 ± 0.010	0.173 ± 0.005
Relative	5.451 ± 0.169	5.249 ± 0.155	5.059 ± 0.370	5.078 ± 0.147
R. Kidney				
Absolute	0.301 ± 0.006	0.310 ± 0.013	0.325 ± 0.010	0.337 ± 0.015
Relative	8.756 ± 0.159	8.811 ± 0.200	9.091 ± 0.210	9.871 ± 0.355**
Liver				
Absolute	1.710 ± 0.056	1.976 ± 0.103*	2.301 ± 0.101**	2.626 ± 0.074**
Relative	49.511 ± 0.863	55.690 ± 1.082**	64.184 ± 1.846**	76.971 ± 1.640**
Lung				
Absolute	0.282 ± 0.014	0.306 ± 0.015	0.304 ± 0.016	0.330 ± 0.010*
Relative	8.173 ± 0.364	8.806 ± 0.539	8.473 ± 0.359	9.624 ± 0.304*
R. Testis				
Absolute	0.086 ± 0.003	0.087 ± 0.004	0.090 ± 0.002	0.086 ± 0.002
Relative	2.516 ± 0.104	2.458 ± 0.077	2.517 ± 0.067	2.526 ± 0.096
Thymus				
Absolute	0.028 ± 0.002	0.033 ± 0.003	0.034 ± 0.003	0.031 ± 0.002
Relative	0.803 ± 0.061	0.901 ± 0.061	0.961 ± 0.065	0.896 ± 0.038
Female				
n	15	8	13	10
Necropsy body wt	31.2 ± 1.2	33.3 ± 2.5	29.0 ± 1.3	28.2 ± 0.7
Heart				
Absolute	0.149 ± 0.003	0.156 ± 0.005	0.145 ± 0.005	0.141 ± 0.005
Relative	4.839 ± 0.152	4.797 ± 0.207	5.048 ± 0.169	5.018 ± 0.148
R. Kidney				
Absolute	0.216 ± 0.005	0.220 ± 0.006	0.214 ± 0.008	0.210 ± 0.004
Relative	7.042 ± 0.223	6.834 ± 0.490	7.434 ± 0.201	7.460 ± 0.225
Liver				
Absolute	1.481 ± 0.058[b]	1.950 ± 0.070**	1.968 ± 0.095**	2.230 ± 0.067**
Relative	47.883 ± 1.262[b]	59.781 ± 2.514**	68.018 ± 1.795**	79.151 ± 2.192**
Lung				
Absolute	0.259 ± 0.011	0.271 ± 0.020	0.282 ± 0.014	0.290 ± 0.015
Relative	8.377 ± 0.363	8.202 ± 0.466	9.846 ± 0.492*	10.279 ± 0.446**
Thymus				
Absolute	0.033 ± 0.003	0.041 ± 0.006	0.032 ± 0.003	0.033 ± 0.002
Relative	1.092 ± 0.098	1.209 ± 0.099	1.098 ± 0.081	1.171 ± 0.070

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).
[b] n=14

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	9	9	10	10
Necropsy body wt	40.6 ± 2.0	38.0 ± 1.6	36.7 ± 1.4	34.9 ± 0.7*
Heart				
Absolute	0.199 ± 0.007	0.193 ± 0.006	0.183 ± 0.008	0.168 ± 0.003**
Relative	4.942 ± 0.176	5.119 ± 0.202	4.988 ± 0.103	4.837 ± 0.106
R. Kidney				
Absolute	0.374 ± 0.015	0.356 ± 0.014	0.350 ± 0.021	0.341 ± 0.011
Relative	9.269 ± 0.252	9.471 ± 0.496	9.500 ± 0.392	9.774 ± 0.283
Liver				
Absolute	2.068 ± 0.122	2.341 ± 0.064	2.613 ± 0.094**[b]	2.662 ± 0.118**
Relative	50.750 ± 1.264	62.467 ± 3.023**	69.852 ± 1.090**[b]	76.306 ± 2.859**
Lung				
Absolute	0.225 ± 0.013	0.234 ± 0.016	0.218 ± 0.007	0.229 ± 0.015
Relative	5.649 ± 0.409	6.214 ± 0.477	5.987 ± 0.207	6.579 ± 0.430
R. Testis				
Absolute	0.090 ± 0.001	0.090 ± 0.002	0.086 ± 0.001	0.075 ± 0.006**
Relative	2.275 ± 0.135	2.395 ± 0.095	2.377 ± 0.092	2.168 ± 0.170
Thymus				
Absolute	0.043 ± 0.005	0.032 ± 0.003	0.032 ± 0.005	0.022 ± 0.002**
Relative	1.040 ± 0.093	0.867 ± 0.112	0.843 ± 0.112	0.616 ± 0.062**
Female				
n	7	9	7	8
Necropsy body wt	34.2 ± 1.1	34.2 ± 2.3	30.9 ± 1.2	28.2 ± 1.7*
Heart				
Absolute	0.162 ± 0.004	0.152 ± 0.007	0.146 ± 0.003	0.134 ± 0.004**
Relative	4.748 ± 0.105	4.510 ± 0.214	4.770 ± 0.165	4.823 ± 0.240
R. Kidney				
Absolute	0.249 ± 0.009	0.237 ± 0.004	0.239 ± 0.007	0.225 ± 0.010
Relative	7.311 ± 0.203	7.104 ± 0.350	7.767 ± 0.241	8.090 ± 0.397
Liver				
Absolute	1.815 ± 0.067	2.115 ± 0.093	2.237 ± 0.058*	2.172 ± 0.120*
Relative	53.126 ± 0.667	63.379 ± 4.158*	72.912 ± 2.566**	77.593 ± 3.227**
Lung				
Absolute	0.205 ± 0.007	0.203 ± 0.010	0.194 ± 0.008	0.232 ± 0.013
Relative	5.999 ± 0.156	6.156 ± 0.513	6.324 ± 0.342	8.433 ± 0.648**
Thymus				
Absolute	0.033 ± 0.003	0.030 ± 0.001	0.030 ± 0.001	0.028 ± 0.003
Relative	0.974 ± 0.070	0.898 ± 0.045	0.980 ± 0.029	0.998 ± 0.072

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	15	15	15	15
Necropsy body wt	47.4 ± 0.8	45.5 ± 1.3	37.0 ± 1.5**	32.9 ± 0.9**
Heart				
Absolute	0.213 ± 0.007	0.202 ± 0.007	0.177 ± 0.004**	0.172 ± 0.005
Relative	4.501 ± 0.128	4.446 ± 0.131	4.849 ± 0.147	5.232 ± 0.125**
R. Kidney				
Absolute	0.307 ± 0.009	0.285 ± 0.008	0.265 ± 0.010**	0.261 ± 0.005**
Relative	6.494 ± 0.209	6.328 ± 0.246	7.235 ± 0.206*	7.974 ± 0.210**
Liver				
Absolute	2.716 ± 0.138	2.725 ± 0.176	2.345 ± 0.124	2.655 ± 0.078
Relative	56.925 ± 2.138	59.197 ± 2.182	63.235 ± 1.176*	80.726 ± 1.092**
Lung				
Absolute	0.284 ± 0.009	0.267 ± 0.017	0.278 ± 0.009	0.280 ± 0.012
Relative	6.031 ± 0.230	5.924 ± 0.427	7.719 ± 0.404**	8.610 ± 0.481**
R. Testis				
Absolute	0.110 ± 0.002	0.111 ± 0.003	0.105 ± 0.002	0.105 ± 0.002
Relative	2.338 ± 0.061	2.459 ± 0.069	2.893 ± 0.110**	3.230 ± 0.113**
Thymus				
Absolute	0.057 ± 0.003	0.055 ± 0.006	0.048 ± 0.002	0.043 ± 0.002*
Relative	1.195 ± 0.052	1.199 ± 0.126	1.310 ± 0.058	1.318 ± 0.046
Female				
n	15	15	14	14
Necropsy body wt	31.4 ± 1.4	28.8 ± 1.0	25.3 ± 0.5**	25.0 ± 0.4**
Heart				
Absolute	0.157 ± 0.003	0.153 ± 0.004	0.151 ± 0.003	0.139 ± 0.003**
Relative	5.105 ± 0.203	5.351 ± 0.111	5.986 ± 0.142**	5.563 ± 0.090
R. Kidney				
Absolute	0.199 ± 0.004	0.193 ± 0.004	0.188 ± 0.004	0.183 ± 0.004**
Relative	6.466 ± 0.222	6.749 ± 0.135	7.450 ± 0.141**	7.341 ± 0.089**
Liver				
Absolute	1.443 ± 0.044	1.721 ± 0.065**	1.782 ± 0.041**	2.064 ± 0.045**
Relative	46.438 ± 0.953	59.841 ± 1.158**	70.375 ± 0.862**	82.671 ± 1.175**
Lung				
Absolute	0.274 ± 0.012	0.281 ± 0.012	0.265 ± 0.013	0.251 ± 0.008
Relative	8.960 ± 0.524	9.931 ± 0.521	10.451 ± 0.474	10.069 ± 0.365
Thymus				
Absolute	0.058 ± 0.002	0.056 ± 0.002	0.054 ± 0.002	0.051 ± 0.002
Relative	1.882 ± 0.100	1.941 ± 0.073	2.125 ± 0.087	2.041 ± 0.060

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid[a]

	0 mg/L	500 mg/L	1,000 mg/L	2,000 mg/L
Male				
n	9	10	9	10
Necropsy body wt	52.3 ± 1.2	46.8 ± 1.7*	35.7 ± 1.2**	35.8 ± 2.0**
Heart				
Absolute	0.263 ± 0.016	0.232 ± 0.011	0.220 ± 0.016*	0.181 ± 0.008**
Relative	5.006 ± 0.235	4.977 ± 0.198	6.165 ± 0.409*	5.150 ± 0.302
R. Kidney				
Absolute	0.376 ± 0.016	0.332 ± 0.014*	0.307 ± 0.008**	0.271 ± 0.011**
Relative	7.196 ± 0.271	7.125 ± 0.248	8.676 ± 0.343**	7.657 ± 0.186
Liver				
Absolute	3.656 ± 0.108	3.274 ± 0.286	2.458 ± 0.060**	2.981 ± 0.144**
Relative	69.979 ± 1.672	68.937 ± 3.757	69.101 ± 1.300	83.735 ± 1.834**
Lung				
Absolute	0.231 ± 0.009	0.240 ± 0.016	0.243 ± 0.030	0.235 ± 0.016
Relative	4.418 ± 0.156	5.183 ± 0.356	6.811 ± 0.832**	6.743 ± 0.593**
R. Testis				
Absolute	0.112 ± 0.003	0.108 ± 0.002	0.102 ± 0.002**	0.102 ± 0.003**[b]
Relative	2.149 ± 0.051	2.339 ± 0.107	2.898 ± 0.122**	2.855 ± 0.149**[b]
Thymus				
Absolute	0.071 ± 0.005	0.114 ± 0.056	0.040 ± 0.004	0.044 ± 0.003
Relative	1.351 ± 0.094	2.381 ± 1.145	1.103 ± 0.102	1.229 ± 0.054
Female				
n	10	9	10	9
Necropsy body wt	43.7 ± 2.5	37.7 ± 2.8*	28.2 ± 0.9**	26.9 ± 0.7**
Heart				
Absolute	0.179 ± 0.004	0.178 ± 0.004	0.153 ± 0.004**	0.150 ± 0.006**
Relative	4.185 ± 0.204	4.899 ± 0.332*	5.450 ± 0.142**	5.589 ± 0.187**
R. Kidney				
Absolute	0.235 ± 0.005	0.234 ± 0.005	0.207 ± 0.004**	0.189 ± 0.005**
Relative	5.505 ± 0.248	6.431 ± 0.378*	7.387 ± 0.237**	7.035 ± 0.219**
Liver				
Absolute	1.854 ± 0.088	2.089 ± 0.091*	2.160 ± 0.078**	2.390 ± 0.055**
Relative	42.847 ± 1.448	56.776 ± 2.618**	76.712 ± 1.960**	88.810 ± 0.984**
Lung				
Absolute	0.224 ± 0.008	0.202 ± 0.007	0.206 ± 0.014	0.241 ± 0.023
Relative	5.203 ± 0.209	5.580 ± 0.405	7.315 ± 0.414**	8.917 ± 0.724**
Thymus				
Absolute	0.052 ± 0.003	0.050 ± 0.003	0.052 ± 0.004	0.047 ± 0.003
Relative	1.200 ± 0.052	1.373 ± 0.095	1.847 ± 0.117**	1.741 ± 0.117**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Dichloroacetic Acid

Dichloroacetic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI) in two lots (05316AR and 11905BU) that were used in the 26- and 39-week dermal studies and the 26- and 41-week drinking water studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Memorial Institute (Columbus, OH) and the study laboratory at Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the dichloroacetic acid studies are on file at the National Institute of Environmental Health Sciences.

Lots 05316AR (a yellow liquid) and 11905BU (a colorless liquid) were identified as dichloroacetic acid by the analytical chemistry laboratory using infrared spectroscopy (IR) and proton and carbon-13 nuclear magnetic resonance spectroscopy (NMR). Lots 05316AR and 11905BU were identified as dichloroacetic acid by the study laboratory using IR All IR spectra were consistent with the structure of dichloroacetic acid, with a reference spectrum, and with literature spectra (*Aldrich*, 1985; *Sigma*, 1986) of dichloroacetic acid. The NMR spectra were consistent with the structure of dichloroacetic acid and with literature spectra (*Aldrich*, 1992). IR, proton NMR, and carbon-13 NMR spectra are presented in Figures G1 through G3.

The purity of lot 05316AR was determined by the analytical chemistry laboratory and the study laboratory using high-performance liquid chromatography (HPLC) and by the analytical chemistry laboratory using acid functional group titration. HPLC was performed using a Prodigy 5 ODS-3 column (150 × 4.6 mm, 5 µm particle size; Phenomenex, Torrance, CA) with ultraviolet detection at 220 nm and a mobile phase composed of A) 15 mM phosphoric acid and B) 30 mM phosphoric acid:acetonitrile (1:1); 100% A to 100% B in 20 minutes, 100% B for 15 minutes to 100% A in 5 minutes, and 100% A for 25 minutes at a flow rate of 1.0 mL/minute. Acid functional group titration was performed by titrating a 4 mg/mL solution of dichloroacetic acid in deionized water with certified 0.1003 N sodium hydroxide on a Metrohm 702 SM Titrino automatic titrator with a Metrohm combined pH glass electrode.

For lot 11905BU, moisture content analysis by Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN). The purity of lot 11905BU was determined by the analytical chemistry using HPLC by the system described for lot 05316AR, by the analytical chemistry laboratory using acid functional group titration by the method described for lot 05316AR, and by the analytical chemistry laboratory using ion chromatography (IC). IC was performed using an Ionpac AS11 column (250 mm × 4 mm; Dionex, Sunnyvale, CA) with suppressed conductivity detection and a mobile phase composed of A) 4 mM sodium hydroxide B) 32 mM sodium hydroxide; 100% A for 9.9 minutes to 100% B in 0.1 minute, 100% B for 13.9 minutes to 100% A in 0.1 minutes, and 100% A for 6 minutes at a flow rate of 1.5 mL/minute.

For lot 05316AR, HPLC at the analytical chemistry laboratory indicated one major peak and three impurity peaks with areas less than 1.0% of the major peak area, a combined impurity peak area of 1.20% of the major peak area, and a purity of approximately 98.8%. HPLC at the study laboratory indicated a purity of 99.9% relative to a frozen reference standard of the same lot. Acid functional group titration indicated a purity of approximately 99.4%. The overall purity of lot 05316AR was determined to be greater than 98.8%.

For lot 11905BU, Karl Fischer titration indicated that a minimal amount of water (0.06%) was present. HPLC at the analytical chemistry laboratory indicated one major peak and no impurity peaks greater than or equal to 0.1% of the area of the major peak and a purity of 100%. HPLC at the study laboratory indicated a purity of 100.3%.

Acid functional group titration indicated a purity of approximately 100.6%. IC indicated one major peak and four impurity peaks with combined peak areas of 0.77% of the major peak area. The overall purity of lot 11905BU was determined to be greater than 99.9%.

Stability studies of a different lot (05703LS) of bulk chemical were performed by the analytical chemistry laboratory using HPLC. HPLC by the system described for purity determination indicated that dichloroacetic acid was stable as a bulk chemical for at least 14 days when stored under a minimal headspace, protected from light in amber glass containers at temperatures up to 60° C. To ensure stability the bulk chemical was stored at room temperature, protected from light in amber glass containers. Stability was monitored by the study laboratory during the 26-, 39-, and 41-week studies. No degradation of the bulk chemical was detected. Reports on the reanalysis of dichloroacetic acid are on file at the National Institute of Environmental Health Sciences.

12-*O*-tetradecanoylphorbol-13-acetate

12-*O*-tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO) in one lot (48H1178) that was used in the 26-week studies in Tg.AC hemizygous mice. Lot 48H1178, a white crystalline powder, was identified as TPA by Research Triangle Institute (RTI; Research Triangle Park, NC) using IR and proton NMR spectroscopy. All spectra were consistent with the structure of TPA.

The purity of lot 48H1178 was determined by RTI using HPLC. HPLC analysis was performed with a Dupont Zorbax Rx C8 column (25 cm × 4.6 mm; Agilent Technologies, Palo Alto, CA), photodiode array detection monitored at 232 nm, and an isocratic mobile phase of water:acetonitrile (10:90) with a flow rate of 1.0 mL/minute. Analysis indicated one major peak and one impurity peak with an area equal to approximately 0.11% of the total integrated peak area. The overall purity of lot 48H1178 was determined to be greater than 99%. The TPA formulations were shown to be stable for at least 6 months.

Acetone

USP-grade acetone was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in two lots (OG0513 and OX0312) that were used in the 26- and 39-week dermal studies. Lots OG0513 and OX0312, clear liquids, were identified as acetone using IR spectroscopy; the IR spectra were consistent with a literature spectrum.

The purity of lots OG0513 and OX0312 was determined using gas chromatography (GC). The analytical system used a 20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport column (2.4 m × 2 mm; Sigma-Aldrich, St. Louis, MO), a flame ionization detector, helium carrier gas flow rate of approximately 30 mL/minute, and an oven temperature program of 40° C for 4 minutes, then 10° C/minute to 170° C. Analysis indicated one major peak and no impurities with areas greater than or equal to 0.1% of the major peak. The overall purity of both lots was determined to be greater than 99.9%.

Preparation and Analysis of Dose Formulations

Dermal Studies

The dose formulations were prepared every 1 to 5 weeks by mixing dichloroacetic acid with deionized water to obtain the required final concentration of dichloroacetic acid (Table G1), adjusting the pH of the solution within a range of six to eight using 20 N sodium hydroxide and 0.1 N hydrochloric acid and adding USP-grade acetone to obtain a water:acetone volume ratio of 1:2. The dose formulations were stored at room temperature in amber glass bottles with Teflon®-lined lids and used within 35 days. TPA formulations in acetone were prepared by RTI and administered by the study laboratory.

Stability studies of a 9.5 mg/mL formulation were performed by the study laboratory using GC. The GC system

used an RTX-5 column (30 m × 0.53 mm, 1.5 µm film thickness; Restek, Bellefonte, PA), a flame ionization detector, helium carrier gas flowing at approximately 10 mL/minute, and an oven temperature program of 50° C for 1 minute, then 12° C/minute to 150° C, then 70° C/minute to 300° C, and then held for 3 minutes. Stability was confirmed for at least 35 days for dose formulations stored in sealed amber glass containers protected from light at room temperature. Stability of dichloroacetic acid was confirmed in formulations stored open to air and light for at least 3 hours; increases in dichloroacetic acid concentration occurred due to evaporation of acetone.

Periodic analyses of the dose formulations of dichloroacetic acid were conducted by the study laboratory using GC by the system described for formulation stability. During the 26- and 39-week studies, dose formulations were analyzed five times; all 12 of the dose formulations were within 10% of the target concentrations (Table G2). Animal room samples of those dose formulations were also analyzed; five of six animal room samples were within 10% of the target concentrations.

Drinking Water Studies

Dose formulations were prepared every 1 to 5 weeks by adding a specified amount of dichloroacetic acid to tap water in a calibrated NALGENE® tank to obtain the required concentration (Table G2). The dose formulations were stored at room temperature protected from light in the NALGENE® tanks in which they were prepared with the lids sealed with Parafilm or tape and used within 42 days after formulation. TPA formulations in acetone were prepared by RTI and administered by the study laboratory.

Stability studies of 10 and 100 µg/mL dose formulations were conducted by the analytical chemistry laboratory using an IC system similar to that described for purity determination. Stability was confirmed for at least 42 days for formulations sealed and protected from light in amber glass or NALGENE® containers stored at 5° C and room temperature.

Periodic analyses of the dose formulations of dichloroacetic acid were conducted by the study laboratory using HPLC. HPLC was performed using a Prodigy 5 ODS-3 column (150 × 4.6 mm, 5 µm particle size; Phenomenex, Torrance, CA), with ultraviolet detection at 220 nm, and a mobile phase composed of A) 15 mM phosphoric acid and B) 15 mM phosphoric acid:acetonitrile (1:9); 100% A for 13.9 minutes at a flow rate of 1.0 mL/minute, 100% B to 100% A in 0.1 minutes at 1.5 mL/minute, 100% B for 5.9 minutes at 1.5 mL/minute 100% B to 100% A in 0.1 minutes at 1.0 mL/minute and 100% A for 5 minutes at 1.0 mL/minute. During the 26- and 41-week studies, dose formulations were analyzed four times; all 12 of the dose formulations for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target concentrations (Table G3). Animal room samples of those dose formulations were also analyzed; five of six animal room samples for Tg.AC hemizygous mice and all six of the animal room samples for p53 haploinsufficient mice were within 10% of the target concentrations.

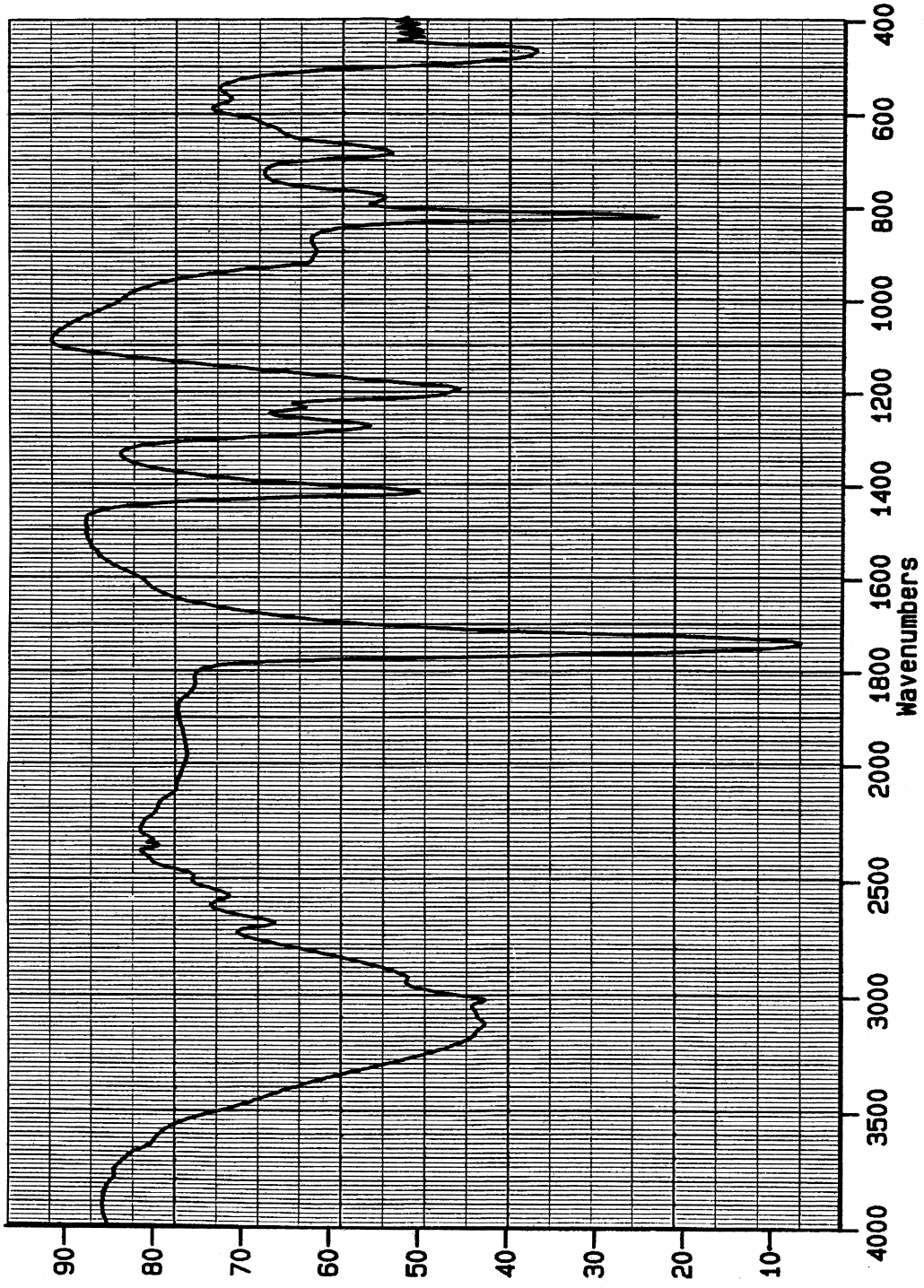

FIGURE G1
Infrared Absorption Spectrum of Dichloroacetic Acid

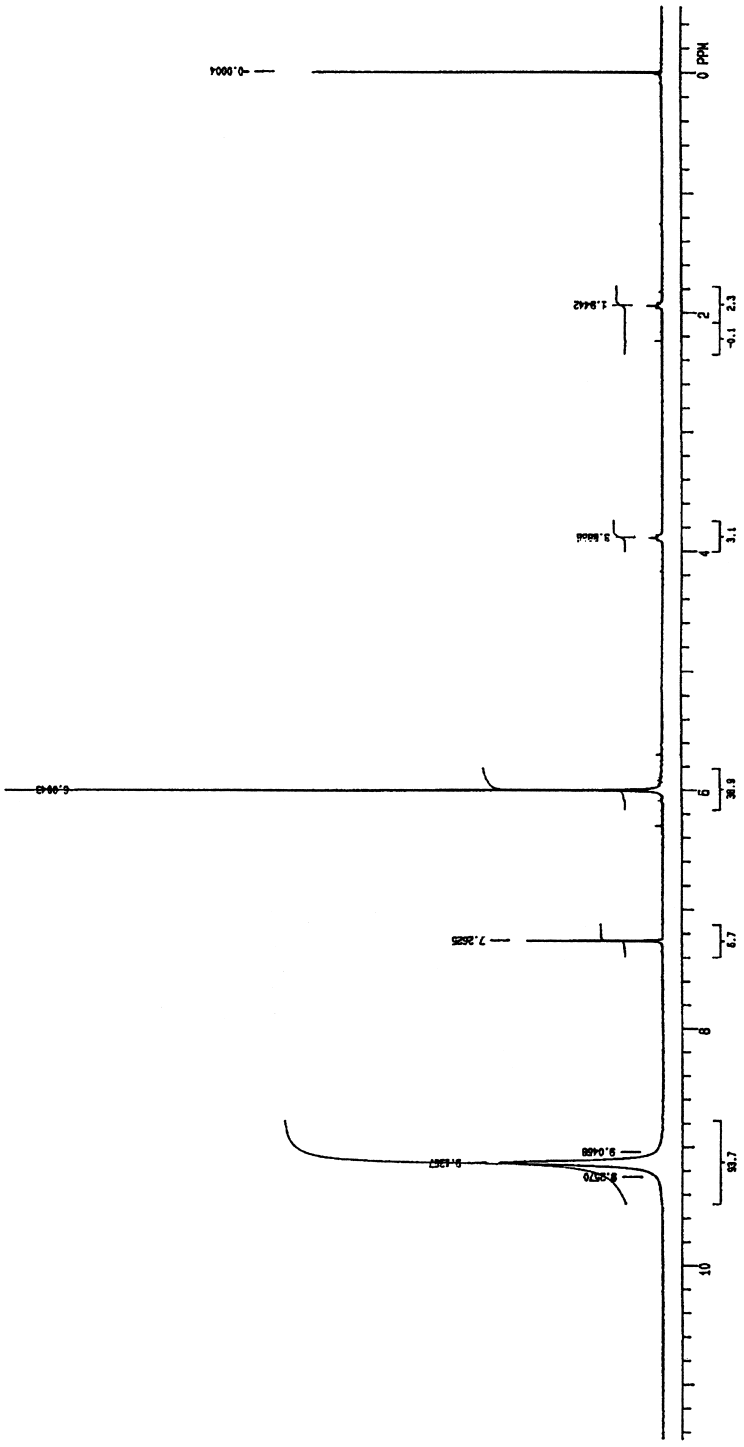

FIGURE G2
Proton Nuclear Magnetic Resonance Spectrum of Dichloroacetic Acid

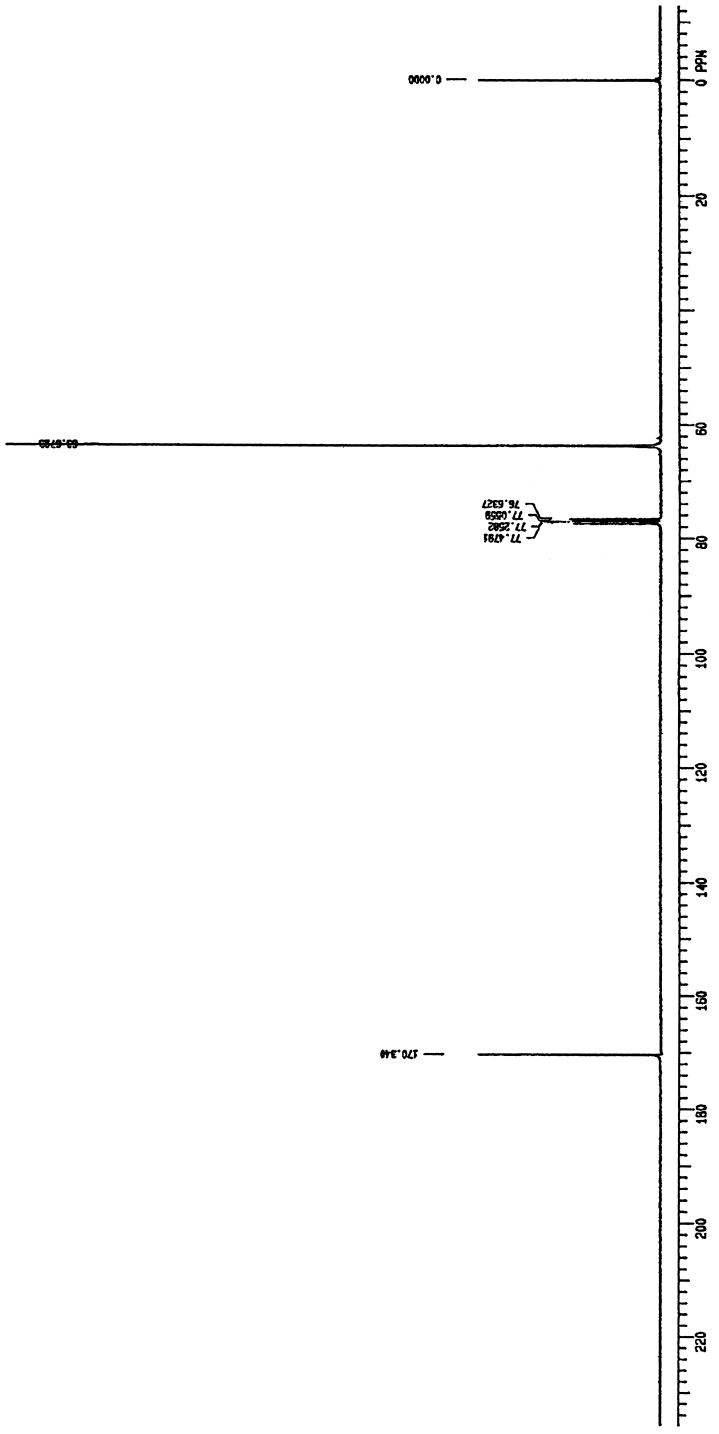

FIGURE G3
^{13}C-Nuclear Magnetic Resonance Spectrum of Dichloroacetic Acid

TABLE G1
Preparation and Storage of Dose Formulations in the Dermal and Drinking Water Studies of Dichloroacetic Acid

Dermal Studies	Drinking Water Studies
Preparation	
Dose formulations were prepared every 1 to 5 weeks by mixing dichloroacetic acid with 1:2 deionized water:USP-grade acetone (Spectrum Chemicals and Laboratory Products, Gardena, CA)	Dose formulations were prepared every 1 to 5 weeks by adding a specified amount of dichloroacetic acid to tap water in a calibrated NALGENE® tank to obtain the required concentration.
12-*O*-tetradecanoylphorbol-13-acetate formulations were prepared by adding the appropriate amount of 12-*O*-tetradecanoylphorbol-13-acetate to acetone.	12-*O*-tetradecanoylphorbol-13-acetate formulations were prepared by adding the appropriate amount of 12-*O*-tetradecanoylphorbol-13-acetate to acetone.
Chemical Lot Numbers	
05316AR 11905BU	05316AR 11905BU
12-*O*-tetradecanoylphorbol-13-acetate: 48H1178	**12-*O*-tetradecanoylphorbol-13-acetate:** 48H1178
Maximum Storage Time	
35 days	42 days
12-*O*-tetradecanoylphorbol-13-acetate: 6 months	**12-*O*-tetradecanoylphorbol-13-acetate:** 6 months
Storage Conditions	
Formulations were transferred to 60 mL amber glass bottles, sealed with Teflon®-lined lids, protected from light, and stored at room temperature.	Formulations remained in the NALGENE® containers in which they were prepared, the lids were sealed with parafilm or tape, protected from light, and stored at room temperature.
12-*O*-tetradecanoylphorbol-13-acetate: Stored in amber glass bottles sealed with Teflon®-lined lids at 5° C.	**12-*O*-tetradecanoylphorbol-13-acetate:** Stored in amber glass bottles sealed with Teflon®-lined lids at 5° C.
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE G2
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice in the 26- and 39-Week Dermal Studies of Dichloroacetic Acid

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
February 2, 2000	February 7, 2000	37.9	38.22	+1
		151.5	155.4	+3
	March 15, 2000[b]	37.9	39.90	+5
		151.5	161.6	+7
February 9, 2000	February 9, 2000	9.48	9.821	+4
	March 15, 2000[b]	9.48	10.78	+14
April 19, 2000	April 20-21, 2000	9.48	9.891	+4
		37.9	35.94	–5
		151.5	149.3	–1
July 10, 2000	July 12-13, 2000	9.48	9.402	–1
		37.9	37.31	–2
		151.5	148.2	–2
October 4, 2000	October 4-5, 2000	9.48	10.24	+8
		37.9	37.52	–1
		151.5	154.3	+2
	November 9, 2000[b]	9.48	10.08	+6
		37.9	36.26	–4
		151.5	159.7	+5

[a] Results of duplicate analyses. Dosing volume=3.3 mL/kg; 9.48 mg/mL=31.25 mg/kg, 37.9 mg/mL=125 mg/kg, 151.5 mg/mL=500 mg/kg.
[b] Animal room samples

TABLE G3
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice and p53 Haploinsufficient Mice in the 26- and 41-Week Drinking Water Studies of Dichloroacetic Acid

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Tg.AC Hemizygous Mice and p53 Haploinsufficient Mice				
January 7, 2000	January 10-11, 2000	500	506.3	+1
		1,000	1092	+9
		2,000	2093	+5
March 27, 2000	March 28-29, 2000	500	520.9	+4
		1,000	1004	0
		2,000	2037	+2
June 19, 2000	June 21-22, 2000	500	511.0	+2
		1,000	1027	+3
		2,000	1980	–1
September 6, 2000	September 7-8, 2000	500	507.7	+2
		1,000	1030	+3
		2,000	2125	+6
Animal Room Samples				
Tg.AC Hemizygous Mice				
January 7, 2000	February 15-16, 2000	500	524.2	+5
		1,000	1067	+7
		2,000	2241	+12
September 6, 2000	October 18-19, 2000	500	512.2	+2
		1,000	1034	+3
		2,000	2077	+4
p53 Haploinsufficient Mice				
January 7, 2000	February 15-16, 2000	500	524.3	+5
		1,000	1068	+7
		2,000	2140	+7
September 6, 2000	October 18-19, 2000	500	508.8	+2
		1,000	1022	+2
		2,000	1983	–1

[a] Results of duplicate analyses

APPENDIX H
WATER AND COMPOUND CONSUMPTION IN THE 26-WEEK AND 41-WEEK DRINKING WATER STUDIES OF DICHLOROACETIC ACID

TABLE H1
Water and Compound Consumption by Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	5.7	22.5	5.2	22.8	113	4.0	23.2	172	2.8	22.8	242
3	5.4	23.7	4.2	24.3	86	3.7	25.1	147	3.0	24.8	239
4	5.4	25.4	4.0	26.3	76	5.1	26.4	191	2.9	26.1	220
5	5.9	26.4	5.0	27.4	91	3.4	27.3	124	2.9	27.0	216
6	5.1	27.1	3.2	27.7	57	3.7	27.4	134	3.3	27.8	240
7	4.9	27.8	4.7	28.2	83	5.1	28.1	181	2.9	27.9	210
8	5.0	28.4	3.5	29.0	60	4.6	29.1	160	4.0	28.5	281
9	4.7	28.8	3.7	29.3	64	5.2	30.0	172	4.1	28.9	283
10	4.8	28.6	5.6	30.2	92	4.3	29.8	144	4.1	28.8	288
11	4.4	29.7	4.1	30.8	66	4.8	30.6	158	4.1	29.9	275
12	4.4	30.8	5.0	31.3	80	4.4	30.8	141	4.6	30.9	296
13	3.9	31.0	5.4	31.6	85	4.3	31.6	137	4.9	31.2	314
14	4.4	31.2	4.8	32.0	74	4.8	31.6	152	4.2	31.3	268
15	4.0	31.5	5.4	32.2	84	5.0	31.6	159	4.1	31.5	259
16	3.9	31.3	5.5	32.5	84	4.7	31.8	148	4.0	31.6	251
17	3.8	31.5	5.4	33.4	81	4.4	31.9	139	3.7	32.1	228
18	4.3	31.7	5.7	33.7	85	4.5	32.3	139	3.4	32.0	211
19	4.3	32.5	5.1	34.7	73	4.2	33.4	124	3.7	33.3	224
20	4.0	32.8	5.0	35.3	70	4.1	32.8	126	3.0	32.8	182
21	4.2	33.2	5.6	35.7	78	3.9	31.7	123	3.9	33.1	238
22	4.1	33.6	5.1	35.7	72	4.5	35.7	125	3.3	33.3	198
23	4.1	33.4	4.0	35.4	56	4.7	35.7	133	3.4	33.9	202
24	3.9	33.8	4.3	35.4	61	4.6	35.9	128	3.0	33.7	181
25	3.9	34.5	4.2	35.5	59	4.4	36.5	120	3.4	34.1	201
Mean for weeks											
2-13	5.0	27.5	4.5	28.2	79	4.4	28.3	155	3.6	27.9	259
14-25	4.1	32.6	5.0	34.3	73	4.5	33.4	135	3.6	32.7	220

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H2
Water and Compound Consumption by Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	5.7	18.9	5.3	19.2	139	4.0	19.6	207	2.9	19.3	298
3	5.3	19.4	6.2	20.5	150	4.6	20.5	223	3.4	20.5	337
4	4.9	21.5	6.5	21.3	152	5.4	21.7	249	3.4	21.2	321
5	5.8	22.3	5.8	22.2	130	4.9	22.2	220	3.7	21.9	337
6	4.3	22.8	5.2	22.2	118	5.0	22.8	221	3.8	21.9	347
7	5.7	22.9	5.4	22.2	122	4.7	23.1	206	3.8	22.4	336
8	5.7	24.0	5.0	23.2	107	4.4	23.7	188	4.2	22.8	370
9	5.5	24.3	5.1	24.3	106	4.8	24.4	197	4.0	23.2	348
10	6.2	24.2	5.7	24.9	115	4.5	24.4	183	4.0	22.6	350
11	6.0	24.6	5.2	24.9	104	4.4	25.1	175	4.1	23.1	350
12	5.8	25.1	5.2	24.7	106	5.2	24.8	208	4.4	23.9	368
13	5.7	25.5	6.4	25.2	127	4.9	24.8	196	4.0	24.1	329
14	5.3	25.5	5.7	25.9	110	5.2	25.2	206	3.5	24.6	283
15	5.1	26.0	5.2	26.2	99	4.6	25.3	182	3.5	24.9	281
16	4.9	26.6	4.5	26.5	85	3.8	25.3	151	3.3	25.0	261
17	5.1	27.3	5.8	27.2	107	4.1	25.3	162	3.3	24.7	269
18	4.9	27.3	4.7	27.4	86	4.4	25.7	172	3.3	24.6	271
19	4.7	27.6	4.8	29.6	81	4.6	25.8	179	3.2	25.2	254
20	5.0	28.0	4.4	30.1	73	3.9	27.2	143	3.1	25.2	250
21	4.7	29.1	4.3	29.7	73	3.6	27.2	131	3.5	25.2	276
22	4.8	29.4	4.2	28.7	73	3.7	27.4	136	3.0	25.9	228
23	4.8	29.9	4.8	30.5	78	3.4	28.2	121	3.1	25.9	237
24	5.0	30.2	3.6	30.5	59	3.4	28.4	119	3.1	26.7	231
25	4.4	30.7	4.5	32.2	70	3.8	28.8	131	3.1	28.0	223
Mean for weeks											
2-13	5.5	23.0	5.6	22.9	123	4.7	23.1	206	3.8	22.2	341
14-25	4.9	28.1	4.7	28.7	83	4.0	26.7	153	3.2	25.5	255

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H3
Water and Compound Consumption by Male Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	6.1	22.7	4.9	22.3	109	3.9	22.8	169	2.7	23.0	233
3	4.4	24.4	5.3	24.8	107	4.2	23.8	177	2.6	24.6	214
4	5.4	25.2				4.8	25.3	190	3.2	25.7	249
5	4.9	27.5				3.3	26.2	124	3.0	26.6	229
6	5.9	28.7				5.2	27.3	191	3.8	27.7	273
7	5.0	28.6				5.2	26.9	192	4.5	28.1	320
8	4.6	29.3	3.7	28.6	65	5.7	28.0	202	5.2	29.1	356
9	5.0	30.3				4.8	28.2	171	4.9	29.6	329
10	6.0	31.2				5.2	28.8	179	4.6	29.3	312
11	5.0	31.6				5.2	29.4	178	4.9	29.9	329
12	5.2	32.5				4.7	29.4	159	4.7	30.1	310
13	4.5	32.7	5.2	31.0	83	5.4	30.3	179	5.1	30.7	335
14	4.3	33.7	5.6	31.7	89	5.0	30.7	163	3.3	30.8	213
15	4.1	33.9				3.6	30.9	117	5.1	31.4	324
16	4.6	34.4	4.6	32.6	71	5.1	31.2	163	3.8	31.6	242
17	4.7	35.2				4.1	31.9	129	4.6	32.8	279
18	4.2	35.6	4.6	33.7	69	4.9	32.1	154	3.4	32.3	212
19	4.2	36.4	7.6	34.1	111	5.3	32.8	162	3.6	32.8	218
20	4.0	36.8	5.7	34.2	83	5.4	33.3	163	3.9	33.1	234
21	4.2	37.5	5.0	34.0	73	5.9	33.7	175	4.0	34.1	237
22	4.0	37.7	5.4	34.2	79	4.0	34.2	118	3.6	34.4	209
23	3.9	37.4	4.4	36.3	61	4.6	34.3	135	3.3	34.2	194
24	3.5	37.9	3.9	35.9	54	4.6	35.1	131	2.9	34.9	168
25	4.4	38.0	4.8	37.1	64	4.5	35.2	129	3.2	35.7	178
26	4.2	38.5	4.9	36.6	67	3.9	35.0	111	3.3	35.8	185
27	4.0	38.4	4.9	38.2	64	4.2	35.2	119	3.6	35.7	202
28	3.8	39.0	4.9	37.4	65	5.0	35.6	140	3.8	35.8	211
29	4.0	38.2	4.0	36.9	54	4.7	34.9	134	3.3	34.9	189
30	4.3	38.7	4.1	37.4	55	4.7	35.4	134	3.1	35.6	172
31	4.3	39.0	4.8	37.6	64	5.1	36.0	141	2.9	35.7	164
32	4.0	39.2	5.1	38.4	66	3.8	36.6	104	3.1	36.1	172
33	6.2	39.9	5.0	39.0	64	4.5	37.0	121	3.2	36.1	177
34	4.5	39.5	5.4	38.1	70	4.5	36.8	122	3.2	35.6	180
35	5.0	40.2	5.0	38.7	65	4.3	36.2	118	3.0	35.2	172
36	5.0	40.4	4.6	37.2	62	5.2	36.4	144	3.1	34.8	179
37	4.1	40.1	3.5	37.1	47	5.0	36.0	140	2.8	34.7	160
38	6.4	40.8	5.3	35.8	74	4.6	36.4	126	4.2	35.0	243
39	4.6	39.6	6.4	37.3	86	4.0	36.4	110	3.9	35.0	221
40	4.2	39.2	5.0	37.4	67	5.0	36.4	138	2.7	34.4	156
Mean for weeks											
2-13	5.2	28.7	4.7	26.7	91	4.8	27.2	176	4.1	27.9	291
14-40	4.4	38.0	5.0	36.3	69	4.7	34.6	135	3.5	34.4	203

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H4
Water and Compound Consumption by Female Tg.AC Hemizygous Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	5.3	19.0	3.0	18.5	81	5.8	19.0	308	3.1	19.3	320
3	6.2	20.2	4.1	20.2	102	5.1	20.3	251	3.7	20.5	358
4	4.9	21.6	5.4	21.0	129	5.4	21.3	253	3.1	21.3	294
5	5.5	22.3				5.9	22.1	265	2.9	22.2	257
6			5.3	23.1	114	7.0	23.0	304	4.1	22.7	360
7			3.6	23.1	78	4.8	23.3	206	3.3	22.8	288
8	6.7	23.8	6.4	23.9	133	5.6	24.2	233	4.2	23.2	359
9	5.8	24.0	5.6	24.1	116	6.0	24.2	248	4.6	23.7	390
10	6.1	24.0	4.5	23.8	94	5.2	24.3	214	3.9	23.2	340
11	6.2	25.1	5.8	24.9	117	5.0	24.5	205	4.0	24.5	323
12	5.6	24.9	4.9	24.3	102	5.5	25.0	221	2.9	24.8	230
13	5.4	26.2	6.0	25.3	119	5.1	25.1	201	3.6	25.4	280
14	4.8	27.3	5.5	25.5	107	4.7	25.6	183	3.9	25.0	311
15	4.9	26.0	4.9	24.5	99	4.8	25.5	187	4.1	24.8	327
16	4.8	26.7	5.9	25.0	117	6.0	26.0	229	3.3	25.5	256
17	4.5	27.3	6.0	25.7	117	5.3	26.4	200	4.6	26.0	353
18	5.1	26.7				4.9	26.1	187	4.0	26.5	305
19	5.1	27.5	5.6	26.9	104	4.6	27.3	168	3.2	26.6	242
20	4.9	28.4	4.7	27.6	86	3.8	27.7	139	4.2	27.2	310
21	4.0	28.1	5.9	27.2	108	4.9	27.5	177	4.2	26.9	309
22	4.3	28.6	5.1	27.6	92	3.8	27.7	139	3.0	27.6	218
23	5.8	29.2	4.5	27.7	82	4.6	28.4	163	3.5	27.9	252
24	5.7	28.4	4.3	28.2	76	4.0	28.4	139	3.3	28.3	233
25	4.8	29.8	4.8	28.4	85	4.8	28.6	168	3.7	28.5	259
26	5.9	30.2	5.1	28.1	91	4.4	27.9	156	3.4	28.9	235
27	5.2	31.3	5.5	29.6	92	4.5	30.0	151	3.2	28.9	223
28	4.4	31.5	4.2	29.6	71	4.8	30.2	159	3.3	29.6	222
29	4.7	31.9	4.2	28.5	73	4.6	29.9	155	3.4	29.7	228
30	4.5	32.8	5.2	30.3	86	4.6	30.1	152	3.0	30.0	198
31	5.1	34.0	4.4	31.0	71	4.7	29.9	158	3.0	30.2	200
32	4.8	33.6	4.6	31.0	74	3.6	31.0	118	2.9	30.3	190
33	4.7	33.3	5.4	31.7	84	4.7	30.9	151	2.6	30.1	172
34	5.0	34.9	4.2	32.2	65	5.8	31.5	185	2.8	30.2	183
35	4.2	35.7	3.9	32.0	60	4.4	30.8	143	2.7	30.2	178
36	3.7	36.1	5.5	32.1	86	4.1	30.4	134	2.9	29.7	194
37	3.8	35.4	4.8	32.1	74	4.6	30.0	152	2.7	27.6	197
38	4.0	36.3	4.7	33.2	71	4.2	29.1	146	3.2	28.7	222
39	3.9	35.1	4.1	33.8	61	3.7	30.3	121	3.6	28.1	258
40	5.2	34.9	4.1	33.6	60	4.8	30.1	158	3.2	27.9	230
Mean for weeks											
2-13	5.8	23.1	5.0	22.9	108	5.5	23.0	242	3.6	22.8	317
14-40	4.7	31.1	4.9	29.4	84	4.6	28.8	160	3.4	28.2	241

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H5
Water and Compound Consumption by Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/mg)	Water (g/day)	Body Weight (g)	Dose (mg/mg)
2	3.3	24.1	3.2	24.0	66	2.8	23.7	118	2.4	22.8	207
3	3.0	25.1	3.0	24.8	61	2.5	23.8	106	2.2	23.6	189
4	3.0	26.4	3.0	26.2	57	2.7	25.4	107	2.1	24.3	176
5	3.1	27.8	3.0	27.6	55	2.6	26.1	100	2.1	25.1	166
6	3.1	28.9	3.0	28.5	52	2.6	26.6	97	2.1	25.5	168
7	3.1	30.7	2.9	29.8	49	2.7	27.9	95	1.9	25.9	148
8	3.1	31.9	2.9	30.7	48	2.6	28.6	93	2.2	26.5	166
9	3.1	33.3	3.1	31.7	48	2.5	29.2	87	2.1	27.1	158
10	3.3	34.7	3.1	33.2	46	2.6	29.8	86	2.1	27.4	152
11	3.2	36.2	2.9	34.0	42	2.5	30.5	82	2.1	27.9	148
12	3.4	37.4	3.1	35.2	44	2.5	31.1	81	2.0	28.4	141
13	3.3	38.6	3.1	36.2	42	2.4	31.1	78	1.9	28.6	136
14	3.6	39.5	3.3	37.1	45	2.5	31.5	79	2.1	29.2	145
15	3.5	40.9	3.0	38.4	40	2.6	32.5	80	2.0	29.4	134
16	3.5	41.4	3.1	38.6	40	2.5	32.4	77	2.3	29.5	156
17	3.8	42.1	3.1	39.8	39	2.5	33.1	76	1.9	29.4	132
18	3.6	43.4	3.0	40.6	37	2.5	33.8	73	2.1	30.5	140
19	3.6	44.6	3.2	41.7	38	2.5	34.6	73	2.0	31.1	132
20	3.6	44.9	3.1	42.2	37	2.5	35.0	71	2.0	31.5	130
21	3.6	45.5	3.0	43.2	35	2.5	35.6	70	2.1	31.8	129
22	3.5	45.8	3.0	43.5	35	2.4	35.8	67	2.1	32.2	129
23	3.7	46.2	3.1	44.0	35	2.4	36.1	66	2.0	32.4	123
24	3.6	46.7	3.2	44.4	36	2.5	36.8	67	2.1	33.1	126
25	3.7	46.9	3.0	44.6	34	2.3	36.6	64	2.0	32.3	127
Mean for weeks											
2-13	3.2	31.3	3.0	30.2	51	2.6	27.8	94	2.1	26.1	163
14-25	3.6	44.0	3.1	41.5	37	2.5	34.5	72	2.1	31.0	134

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H6
Water and Compound Consumption by Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	3.9	19.5	3.8	19.6	96	3.6	19.5	185	2.6	18.5	277
3	4.0	19.9	3.9	20.1	96	3.3	19.7	166	2.3	18.7	246
4	3.9	20.9	4.0	20.6	97	3.4	20.3	169	2.4	19.6	247
5	4.1	21.5	4.4	21.3	103	3.3	20.9	160	2.5	20.3	245
6	4.2	21.7	4.0	21.4	95	3.4	20.9	164	2.5	20.7	244
7	4.0	21.9	3.7	21.7	85	3.4	21.5	156	2.4	21.3	230
8	4.1	23.0	4.1	22.6	90	3.3	22.3	146	2.5	22.0	224
9	3.9	23.3	3.9	22.9	86	3.0	21.3	141	2.4	21.8	216
10	4.2	23.2	3.6	22.3	81	3.6	22.2	162	2.7	22.4	237
11	3.9	23.7	3.9	23.1	84	3.2	22.5	141	2.6	22.2	230
12	4.1	24.5	4.0	23.9	84	3.2	22.9	140	2.5	22.8	218
13	4.1	24.3	3.9	23.9	82	3.1	22.8	137	2.3	22.7	204
14	4.3	24.7	4.5	24.5	91	3.4	23.3	146	2.3	22.8	202
15	4.1	25.3	3.9	24.7	79	3.3	23.6	142	2.5	23.4	216
16	4.6	25.5	4.3	24.6	87	3.2	23.7	134	2.7	23.4	233
17	3.8	24.6	3.7	24.8	74	3.0	23.2	128	2.7	22.6	242
18	4.6	26.8	3.7	25.3	74	3.4	24.1	141	2.4	23.7	204
19	4.2	27.0	3.8	26.3	73	3.2	24.3	131	2.4	23.8	198
20	4.5	27.4	3.7	26.4	69	3.2	24.3	133	2.6	24.3	213
21	4.7	28.1	3.8	27.5	68	3.2	24.6	130	2.4	24.5	196
22	4.5	28.1	3.8	27.3	70	3.3	24.6	133	2.5	24.4	201
23	4.3	29.1	3.8	27.6	68	3.0	24.5	121	2.5	24.4	203
24	4.0	29.2	4.1	27.9	73	3.2	24.6	129	2.4	24.7	195
25	4.2	30.0	3.9	28.5	68	3.0	24.9	122	2.4	24.7	198
Mean for weeks											
2-13	4.0	22.3	3.9	21.9	90	3.3	21.4	155	2.5	21.1	235
14-25	4.3	27.2	3.9	26.3	75	3.2	24.1	133	2.5	23.9	208

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H7
Water and Compound Consumption by Male p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	3.8	24.4	3.0	23.7	63	2.9	23.7	124	2.2	22.8	194
3	3.5	24.9	2.9	24.6	59	2.6	24.0	109	2.1	23.5	179
4	3.7	27.1	3.0	25.6	58	2.9	25.2	113	2.3	24.6	188
5	3.4	28.2	3.1	26.6	58	2.7	25.9	106	2.2	25.4	176
6	3.6	29.4	3.3	27.6	60	2.6	26.4	100	2.3	26.0	173
7	3.4	30.9	3.0	27.9	53	2.7	27.1	99	2.1	26.6	158
8	3.3	32.5	3.1	29.6	53	2.5	27.6	91	2.0	27.3	149
9	3.2	34.0	3.2	30.4	53	2.4	27.8	87	2.0	27.5	143
10	3.4	34.3	3.1	31.6	50	2.7	28.2	94	1.9	28.0	137
11	3.4	36.3	2.8	31.9	44	2.5	28.7	86	1.9	28.0	134
12	3.4	37.2	3.1	33.2	47	2.4	29.4	82	2.0	28.8	140
13	3.6	38.6	3.3	34.4	47	2.5	29.8	84	2.1	29.2	141
14	3.5	39.7	3.5	35.5	49	2.4	30.4	80	2.1	30.0	138
15	3.6	40.9	3.2	35.8	45	2.5	30.6	81	2.0	30.4	134
16	3.5	41.8	3.4	36.9	46	2.5	30.5	83	2.3	30.9	148
17	3.3	42.4	3.4	37.4	45	2.2	30.6	72	2.0	29.7	136
18	3.5	43.4	3.3	38.0	43	2.5	31.0	79	2.3	31.5	144
19	3.5	44.0	3.6	38.8	47	2.5	31.3	81	2.1	32.0	133
20	3.5	45.0	3.4	39.7	43	2.5	31.9	79	2.1	32.4	130
21	3.5	45.5	3.2	40.2	40	2.6	32.5	78	2.1	32.4	131
22	3.5	45.9	3.2	40.5	40	2.4	32.8	74	2.1	32.9	127
23	3.8	46.8	3.5	41.6	42	2.5	33.6	75	2.1	33.7	127
24	3.7	47.3	3.2	42.2	38	2.5	34.5	73	2.2	34.2	127
25	3.8	47.3	3.3	42.1	39	2.4	33.8	70	2.1	33.8	124
26	3.8	47.4	3.2	42.5	38	2.4	33.6	70	2.0	33.4	118
27	3.7	47.7	3.1	42.6	37	2.3	33.8	68	2.0	33.4	120
28	3.9	48.3	3.2	42.7	38	2.4	33.8	70	2.1	33.4	124
29	3.9	48.4	3.3	42.8	38	2.4	33.6	71	2.0	33.2	120
30	4.1	49.1	3.6	43.8	42	2.7	34.3	80	2.4	34.1	141
31	4.2	49.5	3.5	44.1	40	2.8	34.4	81	2.3	34.4	136
32	4.0	50.0	3.5	45.1	39	2.7	35.3	78	2.4	34.8	138
33	4.3	50.6	3.6	45.4	40	2.5	35.5	72	2.2	34.8	127
34	4.3	50.5	3.4	45.9	37	2.3	35.0	67	1.8	34.0	108
35	4.2	50.3	3.3	46.1	36	2.3	34.0	67	1.9	33.5	113
36	4.4	50.9	3.5	46.2	38	2.5	34.3	73	2.0	33.7	120
37	4.3	50.8	3.4	45.5	38	2.3	33.2	69	2.0	33.3	119
38	4.4	51.1	3.6	44.7	40	2.6	32.9	79	2.3	34.1	135
39	3.9	50.0	3.6	45.6	40	2.7	34.2	79	2.5	34.1	148
40	5.0	51.0	4.0	45.9	44	2.5	33.8	73	2.3	35.0	130
Mean for weeks											
2-13	3.5	31.5	3.1	28.9	54	2.6	27.0	98	2.1	26.5	159
14-40	3.9	47.2	3.4	42.1	41	2.5	33.2	75	2.1	33.1	129

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day

TABLE H8
Water and Compound Consumption by Female p53 Haploinsufficient Mice in the 41-Week Drinking Water Study of Dichloroacetic Acid

Week	0 mg/L		500 mg/L			1,000 mg/L			2,000 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
2	4.0	19.5	3.7	19.1	97	3.5	19.1	181	2.6	18.5	284
3	3.7	20.1	3.6	19.6	93	3.2	19.7	162	2.5	19.3	260
4	3.8	21.2	4.2	21.1	99	3.0	20.2	151	2.6	19.9	259
5	3.7	21.9	3.7	22.1	83	3.2	20.8	156	2.5	20.7	237
6	3.9	22.1	3.8	22.4	86	3.9	20.9	187	2.7	21.0	260
7	3.8	22.3	3.4	22.6	76	3.2	20.7	155	2.5	21.1	241
8	3.7	23.2	3.6	23.4	77	3.4	21.6	158	2.8	21.5	257
9	3.7	23.8	3.7	23.6	78	3.3	21.7	151	2.8	21.8	261
10	4.0	23.0	3.8	24.1	80	3.2	21.9	147	2.7	22.0	246
11	3.9	24.6	3.6	24.6	73	3.2	22.1	145	2.6	22.3	236
12	3.8	24.9	3.3	25.4	66	3.3	22.5	146	2.8	22.7	251
13	4.1	25.4	3.8	26.0	72	3.3	22.9	143	2.7	22.8	238
14	4.3	26.4	3.7	26.7	68	3.2	23.0	137	2.6	23.6	218
15	4.3	27.4	3.5	26.8	66	3.1	23.2	135	2.5	23.4	215
16	4.3	27.6	3.7	27.5	67	3.4	23.2	148	2.7	23.7	232
17	3.5	27.1	3.7	27.0	69	3.0	22.7	132	2.8	23.0	239
18	4.2	28.3	4.0	27.5	73	3.7	23.7	158	2.7	23.6	226
19	3.9	29.2	4.0	27.8	71	3.4	24.0	142	2.4	23.9	202
20	3.7	28.8	3.8	28.5	66	3.6	23.8	153	2.6	24.0	214
21	4.3	30.3	3.6	29.4	62	3.4	24.6	140	2.7	24.5	217
22	3.8	30.8	3.4	29.8	58	3.4	24.6	139	2.6	24.6	214
23	4.0	31.6	3.6	30.6	58	3.6	25.1	141	2.6	24.9	212
24	3.9	32.3	3.5	31.1	57	3.3	25.3	132	2.7	25.1	217
25	3.9	32.8	3.7	31.3	60	3.4	25.1	137	2.6	24.7	212
26	3.6	33.2	3.4	30.8	56	3.1	25.3	122	2.6	24.6	211
27	3.7	33.4	3.5	31.7	55	3.1	25.3	121	2.5	24.5	205
28	3.9	34.1	3.2	31.9	51	3.3	25.3	132	2.3	24.6	189
29	3.9	34.9	3.3	32.9	50	3.5	25.1	141	2.2	25.3	177
30	4.1	36.8	3.6	33.8	53	3.5	25.9	136	2.5	25.7	195
31	4.0	37.6	3.6	33.7	53	3.5	25.9	136	2.8	25.3	219
32	4.0	38.5	3.6	34.7	52	3.4	26.3	128	2.8	25.6	219
33	3.9	38.7	3.6	34.6	53	3.5	26.3	134	2.6	25.6	206
34	4.2	39.8	3.6	35.6	50	3.2	26.2	123	2.4	25.3	186
35	4.5	40.1	3.9	36.0	55	3.0	25.9	117	2.3	25.4	181
36	4.3	40.4	4.0	36.8	54	3.2	26.2	123	2.4	25.1	189
37	3.9	40.7	3.5	35.9	49	3.2	26.4	122	2.5	25.5	196
38	4.1	41.3	3.5	35.4	50	3.5	26.8	129	2.4	25.9	185
39	4.2	42.5	3.6	35.8	51	3.8	26.0	147	2.3	25.3	186
40	4.4	43.3	3.8	37.7	50	3.7	27.1	136	2.6	26.1	197
Mean for weeks											
2-13	3.9	22.7	3.7	22.8	82	3.3	21.2	157	2.7	21.1	253
14-40	4.0	34.4	3.6	31.9	58	3.4	25.1	135	2.5	24.8	206

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of dichloroacetic acid consumed per kilogram body weight per day